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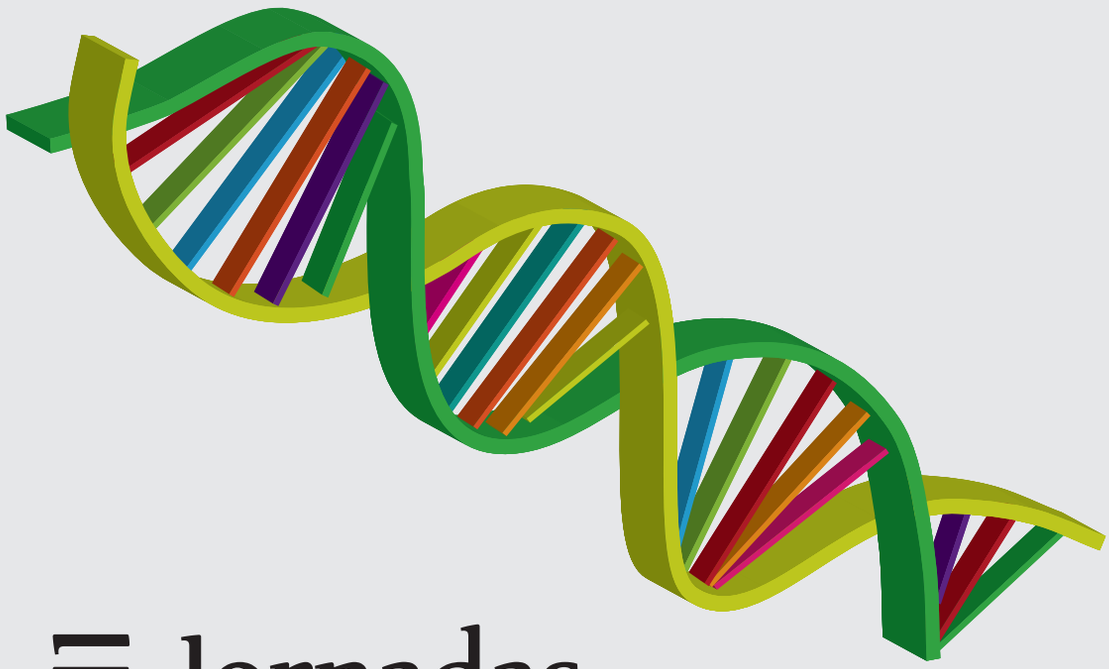

XXXVII Jornadas Portuguesas de Genética

Lisboa
28 a 30 Maio 2012

Universidade Nova de Lisboa

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No presente ano foi lançado ao Departamento de Ciências da Vida, da Faculdade de Ciências e Tecnologia da Universidade Nova Lisboa, o desafio de organizar as XXXVII Jornadas Portuguesas de Genética. Aceitámos esta tarefa, da iniciativa e patrocínio da Sociedade Portuguesa de Genética, com entusiasmo e espírito de missão no sentido da divulgação e intercâmbio de informação cientifica na área da Genética em Portugal.

A comissão organizadora convidou seis cientistas nacionais e estrangeiros que irão proferir palestras plenárias em temas com impacte no panorama actual da genética. O programa científico foi centrado em cinco simpósios temáticos: doenças genéticas e saúde; regulação da expressão genética e vias de sinalização; genética e genómica de plantas; genética e biotecnologia; evolução e genética de populações. Para consubstanciar estes simpósios, foram submetidos 88 resumos, dos quais 30 foram seleccionados para comunicação oral pela comissão científica, e os demais propostos para apresentação em painel.

No sentido de incentivar a participação os jovens cientista e promover a qualidade na investigação, a Sociedade Portuguesa de Genética e a comissão organizadora atribuirão dois prémios, cuja designação pretende homenagear professores e investigadores portugueses que se distinguiram na área de Genética:
Prémio Prof. Doutor Luís Archer - para o melhor trabalho apresentado sob a forma de painel por jovem investigador (primeiro autor);
Prémio Prof. Doutor Amândio Sampaio Tavares - para o melhor trabalho apresentado como comunicação oral por jovem investigador (primeiro autor).

O número de participantes das jornadas ronda os 200, provenientes de diferentes Universidades e Instituições científicas de diversos pontos do País, sendo de realçar a elevada adesão de estudantes de licenciatura e mestrado.

Durante a cerimónia inaugural será prestada homenagem ao Professor Doutor Luís Archer (1926 – 2011). Figura notável e singular, contribuiu decisivamente para o desenvolvimento das Ciências da Vida em Portugal, em particular nas áreas de Genética Molecular e Bioética.
Criou e chefiou o Laboratório de Genética Molecular no Instituto Gulbenkian da Ciência, onde foi investigador durante 20 anos (1971-1991), ao mesmo tempo que leccionou em Portugal e no estrangeiro. Organizou os primeiros cursos monográficos em Portugal, nacionais e internacionais, nas suas áreas de competência. Foi o introdutor em Portugal da investigação em Genética Molecular Microbiana. Foi professor visitante do MIT (EUA, 1973) e ensinou Genética Molecular em muitas Faculdades portuguesas, nomeadamente no Instituto de Ciências Biomédicas da Universidade do Porto, na Faculdade de Ciências da Universidade de Lisboa, e na Faculdade de Ciências e Tecnologia da Universidade Nova de Lisboa (1979-1996), de que era professor catedrático jubilado. Foi presidente da Sociedade Portuguesa de Genética durante dois mandatos e Presidente do Conselho nacional de Ética para as Ciências da Vida, de (1996-2001). Foram-lhe atribuídos diversos prémios e foi alvo de numerosas homenagens promovidas por instituições tanto do mundo científico como académico, sendo de destacar o Doutoramento Honoris Causa atribuído pela Universidade Nova de Lisboa e a Homenagem Nacional organizada pela Sociedade Portuguesa de Genética em 2006. Em 1991, fora agraciado pelo Presidente da República com a Grã-Cruz da Ordem de Sant'lago da Espada.
Com reconhecidas características de excepcional pedagogo, foi um catalisador de entusiasmos e revelador de vocações. A sua contribuição para a percepção pública da importância da Genética Molecular e Engenharia Genética na actividade Humana foi decisiva.

Sejam bem vindos às XXXVII Jornadas Portuguesas de Genética.

Isabel de Sá Nogueira
Presidente da Comissão Organizadora das XXXVII Jornadas Portuguesas de Genética
Departamento de Ciências da Vida
Faculdade de Ciências e Tecnologia
Universidade Nova de Lisboa

Maio 2012

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SPG

Programme Schedule

	Monday, May 28	Tuesday, May 29	Wednesday, May 30	
9.00		**Plenary lecture 2**	**Plenary lecture 5**	9.00
9.15		**Margarida Amaral**	**Chris Hittinger**	9.15
9.30		BioFIG, FC/UL	U. Wisconsin, USA	9.30
9.45				9.45
10.00		**Symposium 2**	**Symposium 4**	10.00
10.15				10.15
10.30		**Coffee break**	**Coffee break**	10.30
10.45		**Posters**	**Posters**	10.45
11.00	**Reception**			11.00
11.15	**and Registration**	**Symposium 2**	**Symposium 4**	11.15
11.30		**Gene Regulation and**	**Genetics and**	11.30
11.45		**Signalling Pathways**	**Biotechnology**	11.45
12.00				12.00
12.15				12.15
12.30		**Lunch**	**Lunch**	12.30
12.45		**Posters**	**Posters**	12.45
13.00				13.00
13.15				13.15
13.30				13.30
13.45				13.45
14.00	**Opening session**			14.00
14.15		**Plenary lecture 3**	**Plenary lecture 6**	14.15
14.30		**Claudio Sunkel**	**Nuno Ferrand**	14.30
14.45	**Plenary lecture 1**	IBMC, UPorto	CIBIO, UPorto	14.45
15.00	**Herminia de Lencastre**			15.00
15.15	ITQB/UNL, U. Rockfeller USA	**Symposium 3**	**Symposium 5**	15.15
15.30		**Plant Genetics**	**Evolution and**	15.30
15.45	**Coffee break**	**and Genomics**	**Population Genetics**	15.45
16.00	**Posters**			16.00
16.15	**Symposium 1**	**Coffee break**	**Coffee break**	16.15
16.30	**Genetic Disorders**	**Posters**	**Posters**	16.30
16.45	**and Health**			16.45
17.00		**Symposium 3**	**Symposium 5**	17.00
17.15				17.15
17.30	**Welcome cocktail**	**Plenary lecture 4**	**Awards and**	17.30
17.45	**Assembleia Geral SPG**	**Wanda Viegas**	**Closing Session**	17.45
18.00		CBAA, ISA/ UTL		18.00
18.15				18.15
18.30				18.30
18.45				18.45
19.00				19.00
19.15				19.15
19.30				19.30

Scientific Programme

Monday, May 28

9.00	Reception and Registration
14.00	Opening Session
14.45	Plenary 1 **H. de Lencastre**, ITQB/UNL, U. Rockefeller, USA “Methicillin resistance in Staphylococcus aureus: mechanisms and evolution”
15.45	Coffee Break Posters
16.15	Symposium 1 Genetic Disorders and Health **S. Lourenço** Classical Galactosemia: First intronic variation characterized as disease-causing mutation
16.30	**I. Silva** Genotype and phenotype variation of *Burkholderia multivorans* sequential isolates during long-term colonization of the airways of cystic fibrosis patients
16.45	**M. Reverendo** Zebrafish as a model system of proteotoxic stress induced by mRNA mistranslation
17.00	**J.M. Xavier** Gene Expression and disease association analyses implicate EREG-AREG and NRG1 in susceptibility to Behçet’s Disease
17.15	**A.C. Adrião** Zebrafish as a model to unveil and study skeletal phenotypes related to human MEF2C mutations
17.30	Welcome cocktail
17.45	Assembleia Geral SPG

Tuesday, May 29

9.00	Plenary 2 **M. Amaral**, BioFIG, FC/UL "From siRNA Screens to Drugs: Illuminating the Cell to Understand Cystic Fibrosis"
10.00 10.15	Symposium 2 Gene Regulation and Signaling Pathways **A.S. Ferreira** Involvement of the BceF tyrosine kinase in stress response, biofilm formation and virulence in *Burkholderia cepacia* IST408 clinical isolate **B. Costa Gomes** Epigenetic Regulation of microRNAs Expression in Breast Cancer
10.30	Coffee Break Posters
11.15 11.30 11.45 12.00 12.15	Symposium 2 Gene Regulation and Signaling Pathways **J. Menino** Gene silencing of the negative regulator of inorganic sulfur assimilation SconC affects virulence of the fungal pathogen *Paracoccidioides brasiliensis* **R.G. Martinho** Tissue specific requirements of *Drosophila* NTC complex in pre-mRNA splicing and transcriptional elongation **J. Rezende-Gomes** Functional analysis of mating genes of the dimorphic fungal pathogen *Paracoccidioides brasiliensis* **M. Saramago** Bacteria can silence genes too: RNase III and small RNAs seek and destroy outer membrane transcript **A. Henriques** Morphodynamics of spore differentiation in relation to pathogenesis in *Clostridium difficile*
12.30	Lunch Posters
14.15	Plenary 3 **C. Sunkel**, IBMC, UPorto "The role of the mitotic checkpoint in chromosome segregation and tumorigenesis"
15.15 15.30 15.45 16.00	Symposium 3 Plant Genetics and Genomics **H.S. Pereira** Rye supernumerary (B) chromosomes may confer heat tolerance during meiosis **R. Diz** Analysis of heat-stress induced genomic modifications in retrotransposon related sequences in *Vitis vinifera* **A. Milhinhos** Thermospermine - a small polycation with large impact in vascular development of poplar **E. Veríssimo** DNA sequence analyses of Phytoene Synthase (Psy) gene in Portuguese durum wheat varieties
16.15	Coffee Break Posters
17.00 17.15	Symposium 3 Plant Genetics and Genomics **F. Reis** Fungal community of chestnut grove: influence of *Hypholoma fasciculare* **A. Soares** Microsatellite analysis of putative grapevine berry colour mutants
17.30	Plenary 4 **W. Viegas**, CBAA, ISA/UTL "Selfish chromosomes: does evolutionary endurance occur without benefits?"

Wednesday, May 30

9.00	Plenary 5 **C. Hittinger**, U. Wisconsin, USA "Surprises in *Saccharomyces* genetic diversity: Evolutionary tales from a budding model genus"
10.00 10.15	Symposium 4 Genetics and Biotechnology **A. Bezerra** Genetic code engineering generates genetic and phenotypic diversity in the human pathogen *Candida albicans* **J. Sá-Pessoa** Uncovering the Short-Chain Fatty Acids transporters in *Escherichia coli*
10.30	Coffee Break Posters
11.15 11.30 11.45 12.00	Symposium 4 Genetics and Biotechnology **M. Cordeiro** Optimisation of Spectral Codification coupled to SBE for genotyping **F. Pereira** A protein required for efficient host colonization by *Clostridium difficile* is an abundant component of the spore surface layers **S. Correia** Vancomycin-Resistant *Enterococci* from Portuguese hemodialysis patients: molecular characterization of antimicrobial resistance, virulence and clonality **A.E. Simões** Cetuximab as a delivery tool for small non-coding RNAs to colon cancer
12.15	Lunch Posters
14.15	Plenary 6 **N. Ferrand**, CIBIO/*InBIO*, UPorto "The genomics of rabbit domestication"
15.15 15.30 15.45 16.00	Symposium 5 Evolution and Population Genetics **G. Seixas** Dengue Vector in Madeira: geographical origin and insecticide resistance **A.T. Avelar** Chromosome structure is a selectable trait **M. David-Palma** Plant associations, extreme diversifying selection and phylogeography of astaxanthin-producing yeasts **F. Azancoth** RAGE T-374A polymorphism as a protective genetic factor for diabetic retinopathy and macular edema
16.15	Coffee Break Posters
17.00 17.15	Symposium 5 Evolution and Population Genetics **J. Cipriano** Evidences from a preliminary molecular study of the probable existence of a native population of Scots Pine (*Pinus sylvestris L.*) in Portugal **J. Vieira** Are all mth-like genes involved in lifespan determination?
17.30	Awards and closing Session

Plenary Lecture 1

Coordinator: Isabel Sá-Nogueira (CREM, FCT/UNL)

PL1

Methicillin resistance in *Staphylococcus aureus*: mechanisms and evolution

H. de Lencastre[1,2]
[1] Laboratory of Molecular Genetics, Instituto de Tecnologia Química e Biológica (ITQB), Universidade Nova de Lisboa, Oeiras, Portugal [2] Laboratory of Microbiology, The Rockefeller University, New York, USA.

The historically first isolates of MRSA already carried resistant factors to several antibiotics (penicillin, streptomycin and tetracycline and often to erythromycin as well) that have been used in clinical practice against *S. aureus* infections prior to the introduction of oxazolin-penicillins, such as methicillin. The methicillin resistance determinant *mecA* encodes a low affinity penicillin binding protein – PBP2A – which is assumed to "take over" the cell wall synthetic transpeptidase (TPase) functions of the four native PBPs of *S. aureus* when these become inactivated by the antibiotic in the medium. How this surrogate TPase can generate the phenotype of high-level resistance for the bacterium is much less clear. Virtually all early isolates of MRSA exhibit the peculiar heterogeneous phenotype in which the great majority of the cells in a culture exhibit only modest levels of resistance. It is only in some, rare MRSA strains such as MRSA strain COL, that extremely high-level and homogeneous resistance is shared by all cells of the culture. Tn*551* mutagenesis has identified over 25 genes in the background of strain COL each one of which is essential for the expression of high-level resistance. The profound influence of these so called auxiliary genes has lead to the conceptually novel proposal that the methicillin resistant phenotype is the product of a stress response pathway in which several background genes have to cooperate with *mecA* to produce the resistant phenotype. The successful integration of this "foreign" gene into the genome of *S. aureus* is demonstrated by experiments that show that PBP2A can also function in the absence of antibiotic in an *S. aureus* mutant with defective transpeptidase function in the native PBP2. In such cells, PBP2A becomes an essential gene for the survival and continued growth of the bacteria. Further evidence for the "domestication" of PBP2A into the genome of *S. aureus* is indicated by experiments which show that the transcription of *mecA* – the genetic determinant of PBP2A – is modulated along with hundreds of other *S. aureus* genes by the level of transcription of *murF,* a key determinant in an early stage of cell wall biosynthesis. Contrary to "paying" a fitness cost for antibiotic resistance, some epidemic clones of MRSA continue to spread globally not only in hospitals but in the community as well and remain one of the most important challenges to the control of bacterial infections in our era.

Plenary Lecture 2

Coordinator: Pedro Baptista (CIGMH, FCT/UNL)

PL2

From siRNA Screens to Drugs: Illuminating the Cell to Understand Cystic Fibrosis

M.D. Amaral[1,2]
[1] University of Lisboa, Faculty of Sciences, BioFIG - Centre for Biodiversity, Functional and Integrative Genomics, Dept Chemistry and Biochemistry,
[2] Centre of Human Genetics, National Institute of Health, Lisboa, Portugal

The information of complete genome sequences and the identification and systematic cloning of human cDNAs provide the challenging opportunity to analyse the complexity of biological processes on a large scale. However, these techniques have limitations, not least of which is their lack of demonstrating a functional involvement of the molecules identified.
Recent advances in automated fluorescence scanning microscopy and image processing allow the application of complete genome knowledge in large-scale screening applications with so far unmatched functional information at the single cell or sub-cellular level. Indeed, in combination with genome-wide/ high-content (HC) small-interference RNA (siRNA) or cDNA over-expression strategies, such microscopy-based applications hold promise to help revealing comprehensively the regulatory networks underlying several cellular processes, such as membrane traffic, in intact cells [1].
The application of cutting-edge microscopy-based screening technology to study traffic and function of two CF-related membrane proteins by siRNA approach, namely CFTR and ENaC will be described. For CFTR, novel constructs (wt- and F508del) were generated, namely bearing both a N-terminus fused fluorescent tag (mCherry) and a Flag epitope tag located at CFTR 4th extracellular loop. These two tags allow determining on each individual cell the fraction of expressed CFTR which is residing in the cell membrane [2]. For ENaC, a functional live-cell assay was selected, based on the activity of ENaC as sodium channel and using the FLIPR membrane potential (FMP) voltage-sensitive fluorescent (blue) dye in combination with the specific ENaC-blocker amiloride [2].
Data will be presented from ongoing work regarding the application of these assays in the context of high-content siRNA screens to identify genes/proteins affecting the traffic of CFTR and function ENaC. In these screens, three high-content libraries of human siRNAs (Ambion) are used, namely: 1) the "kinome" library, targeting 710 different kinase genes; 2) the "secretome" library, targeting 1552 genes involved in the trafficking of a temperature sensitive membrane transport marker, tsO45G; and 3) and the "druggable" targeting different 5940 genes of relatively well known function.
[1] Erfle H et al (2007) *Nat Protoc* **2**: 392-399.
[2] Almaça et al (2011) *Methods Mol Biol* **742**, 249-64.

Plenary Lecture 3

Coordinator: Pedro Baptista (CIGMH, FCT/UNL)

PL3

The role of the mitotic checkpoint in chromosome segregation and tumorigenesis

S. Morais-da-Silva[1], *C.E. Sunkel*[1,2]
[1] Instituto de Biologia Molecular e Celular, Universidade do Porto, Portugal.
[2] Instituto de Ciências Biomédicas de Abel Salazar, Universidade do Porto, Portugal.

Cell proliferation is fundamental for development and to maintain tissue homeostasis. During division it is essential that cells maintain genomic stability since abnormal chromosome segregation causes aneuploidy, a phenomena that has been closely associated with tumorigenesis. Cell division is a highly coordinated process in which different molecular events must take place correctly in time and space. To prevent chromosome missegregation cells have developed surveillance mechanisms or checkpoints that monitor the execution of different events and delay the initiation of subsequent ones in case of errors. Loss of checkpoint control will increase genomic instability eventually causing loss of cycle control and cell transformation. However, whether chromosome missegregation is a cause or a consequence of cell transformation is not yet known. To address this question we have developed in Drosophila a model for tumorigenesis in which we can deplete proteins involved in the Spindle Assembly Checkpoint (SAC) and then ask which other condition is required for tumour formation. We have determined that depleting proteins such as Mad2, BubR1 or Bub3 and at the same time blocking apoptosis by expressing p35 or DIAP1 results in cells that undergo unrestrained cell proliferation with significant chromosome instability which in all respect resemble tumour cells. We have also used microarray analysis to explore the transcriptional profile of these cells which has allowed us to identify a number of essential molecular players that when depleted result in either suppression or enhancement of the tumorigenic phenotype. Moreover, depletion of important proteins required for mechanical attachment of microtubules to kinetochores, which can also induce chromosome instability does not result in tumour formation even if apoptosis is inhibited. These results suggest that SAC proteins behave like tumour suppressors independently of their role in the SAC.

Plenary Lecture 4

Coordinator: Paula Gonçalves (CREM, FCT/UNL)

PL4

Selfish chromosomes: does evolutionary endurance occur without benefits?

W. Viegas[1]
[1] Secção de Genética, Centro de Botânica Aplicada à Agricultura, Instituto Superior de Agronomia,Technical University of Lisbon, Tapada da Ajuda, 1349-017 Lisbon, Portugal

B chromosomes (Bs), defined as supernumerary and dispensable chromosomes, were discovered just over one century ago. Since their discovery, Bs have been identified in a variety of fungi, plant, and animal out crossing species, with species specific DNA sequences and structural organization. Although Bs generally lack genes, they control their own drive process which leads to their survival in natural populations through a non-mendelian mode of transmission. Bs are also a major source of intraspecific variation in nuclear DNA amounts, with population equilibrium for their frequencies being determined by a balance between their accumulation and harmful effects. From a genetics point of view, the absence of Bs from some individuals and their presence in variable numbers in others poses a huge challenge for understanding these chromosomes. Rye (Secale cereale) has provided a number of important insights to B chromosome biology and genetics. Rye Bs are approximately 850 Mb, representing 5.5% of the diploid A genome, organized in both heterochromatic and euchromatic domains. Initially, most phenotypic effects of rye Bs were associated with their negative impact on fertility and grain quality. More recent cytological studies demonstrated that Bs modulate several endophenotypes causing alterations in nuclear chromatin organization.

Rye B DNA sequence analysis has shown that these are mostly composed of repeated DNA sequences common to the As with exception of the subterminal domain which is specially enriched in two repetitive sequence families, namely D1100 and E3900. This B terminal domain has a major role on sister-chromatids adhesion during the first pollen division, guaranteeing Bs preferential transmission to the generative nucleus. Interestingly, chromatin organization of this domain presents an unusual combination of apparently conflicting histone modifications and is preferentially decondensed during meiosis, proportionally with B number. A more in-depth analysis of the E3900 sequence has shown that this sequence is present in a complete 3900 bp as well as a 2700 bp truncated form. These E3900 related sequences accumulate in approximately 100 copies on Bs and are represented in 1-3 copies on A chromosomes in rye as well as other cereals. Interestingly, both these sequence variants are differentially expressed in a tissue and developmental specific manner in plants with and without Bs. Recent results demonstrated that E3900 related transcripts are markedly up-regulated in heat stressed +B plants. Relevantly, a significantly lower proportion of abnormal meiocytes was observed in heat stressed plants with Bs. The hypothesis that rye Bs may have a role in heat tolerance during meiosis may explain their evolutionary endurance.

Plenary Lecture 5

Coordinator: Paula Gonçalves (CREM, FCT/UNL)

PL 5

Surprises in *Saccharomyces* genetic diversity: Evolutionary tales from a budding model genus

Chris T Hittinger[1]
[1] University of Wisconsin-Madison, Laboratory of Genetics, Madison, USA

The budding yeast *Saccharomyces cerevisiae* is one of the most important model species in molecular genetics, but its relatives and evolution have remained obscure until recently. Discoveries from Portugal to Japan to Patagonia have revealed that *Sacccharomyces* are remarkably diverse, both metabolically and genetically, and include at least eight biological species and more than a dozen distinct populations and lineages. We recently showed how the newest member of the genus, *Saccharomyces eubayanus*, hybridized with *S. cerevisiae* ale-brewing yeast to form the allopolyploid yeast responsible for brewing lagers. *S. eubayanus* and its sister species are both more diverse than *S. cerevsiae* and maintain distinct niches in sympatry. Surprisingly, some strains in this lineage contain a functional Dicer gene, despite the fact that they lack the Argonaute gene essential for RNA interference. Genetically removing Dicer in one species affects the expression of dozens of genes, so Dicer may play an unexpected role in gene regulation that does not depend on Argonaute. Finally, a Eurasian species with diverse carbon metabolism has even shown how population structure and strong natural selection can maintain a novel type of complex genetic variation for millions of years. Laboratory strains and high quality genome annotations are now allowing the entire genus to be investigated as unrivaled models of molecular genetics and evolutionary genomics.

Plenary Lecture 6

Coordinator: Isabel Sá-Nogueira (CREM, FCT/UNL)

PL 6

The genomics of rabbit domestication

Nuno Ferrand[1]
[1] CIBIO/*InBIO*, Universidade do Porto. Departamento de Biologia, Faculdade de Ciências do Porto.

For thousands of years humans shaped the genetic and phenotypic composition of several organisms by transforming wild species into domesticated forms. From this close association, domestic species emerged as important models in biomedical and fundamental research, in addition to their intrinsic economical value. The domestic rabbit is no exception but few studies have investigated the impact of domestication across its genome. In the last few years we have been studying patterns of genetic structure in domestic rabbits and quantifying the amount of genetic diversity lost during the process of domestication. Combining microsatellite genotyping, partial genome sequencing and SNP analyses, we first found that both the initial domestication event and the subsequent process of breed formation showed similar losses of genetic diversity, suggesting that corresponding bottlenecks were of equivalent magnitude. Despite the short time elapsed since breed diversification, we uncovered a well-defined breed structure in domestic rabbits. However, in contrast with other domesticated species, we failed to detect deeper levels of structure, probably as a consequence of a recent and single domestication event. Finally, we found strong signs of artificial selection in genomic regions associated with a variety of different traits, suggesting that the rabbit may offer a unique model in investigating the molecular basis of phenotypic evolution.

Symposium 1
Genetic Disorders and Health

Coordinators: Leonor Cancela (CCMAR, UAlg) and Peter Jordan (BioFIG, INSA)

OC1

Classical Galactosemia: First intronic variation characterized as disease-causing mutation

S.P. Lourenço[1], *A.I. Coelho*[1], *M.J. Silva*[1], *I. Tavares*[1], *J.B. Vicente*[1], *I. Rivera*[1]
[1] iMed.UL, Department of Biochemistry and Human Biology, Faculty of Pharmacy, University of Lisbon, Portugal;
Presenting author's e-mail: s.lourenco@campus.ul.pt

Classical Galactosemia is an Inborn Error of Metabolism (IEM) caused by deficient activity of galactose-1-phosphate uridylyltransferase (GALT). This enzyme is encoded by the GALT gene, which is located on chromosome 9p13, spans about 4 kb of genomic DNA and is structured into 11 exons. Like many other recessive metabolic disorders, Classical Galactosemia displays a great allelic heterogeneity with more than 200 variations described until now, most of them being missense ones. Mutational analysis of 27 Portuguese patients confirmed the Q188R as the prevalent molecular defect (≈60%), and revealed the intronic variation c.820+13a>g (IVS8+13a>g) as the second most frequent mutation, accounting for 12.5% of mutant alleles. This intronic variation is believed to affect pre-mRNA splicing, and therefore to be a disease causing mutation since it has never been identified in controls and was only found in patients either in homozygous or compound heterozygous state. In this study, we investigated the pathogenic mechanism of c.820+13a>g mutation through functional splicing assays using a minigene approach. The pSPL3 vector containing either the genomic wild-type or the mutant fragments was transfected into COS-7 cell line. 24h after transfection, RNA was purified and amplified by RT-PCR. Reaction products were separated by agarose gel electrophoresis and analyzed by direct sequencing. The results clearly showed that c.820+13a>g favors the next GT dinucleotide (c.820+14_15) to be used as a new 5' splice donor site, leading to alternative splicing. This mutation will cause the inclusion of the first 13 nucleotides of intron 8 in the coding sequence, thus inducing a frameshift. Though several intronic mutations, putatively affecting splicing, have been identified in galactosemic patients, c.820+13a>g is the first one whose molecular mechanism was elucidated. In conclusion, this intronic variation is indeed a disease-causing mutation, affecting patients' splicing pattern which supports the growing evidence that intronic variations/substitutions might be misclassified and that the extent of splicing mutations must be underestimated.

OC2

Genotype and phenotype variation of *Burkholderia multivorans* sequential isolates during long-term colonization of the airways of cystic fibrosis patients

I. Silva[1], *A. Ferreira*[1], *J. Becker*[2], *A. Tavares*[1], *L. Moreira*[3]
[1] Institute for Biotechnology and Bioengineering, Centre for Biological and Chemical Engineering, IST, Av. Rovisco Pais, 1049-001 Lisbon, Portugal;
[2] Instituto Gulbenkian da Ciência, Rua da Quinta Grande 6, 2780-156, Oeiras, Portugal;
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Cystic fibrosis is the most common autosomal recessive disorder in Caucasians and is caused by mutations in the CF transmembrane conductance regulator gene (CFTR). CF affects various organs including the respiratory tract and CF patients suffer from chronic lung inflammation frequently caused by chronic bacterial infections. *Burkholderia cepacia* complex (Bcc) bacteria are opportunistic pathogens infecting CF patients. These bacteria are extremely difficult to eradicate from CF lung and recent studies linked Bcc long-term infection of CF patient airways to the emergence of phenotypic variation (Zlosnik et al. 2008). Here we report the study of two *Burkholderia multivorans* clonal isolates displaying different mucoid morphotypes from a chronically infected CF patient. Genome sequencing showed the deletion of a region with more than 100 kb in the nonmucoid isolate D2214. In addition, expression profiling of mucoid D2095 and nonmucoid D2214 isolates using custom *Burkholderia* microarrays revealed decreased expression of genes encoding products related to virulence-associated traits and metabolism in D2214 (Silva et al. 2011). *B. multivorans* D2214 showed no exopolysaccharide production, lower motility and chemotaxis, and more biofilm formation, particularly under microaerophilic conditions, than the clonal mucoid isolate D2095. Also, D2214 showed attenuated virulence than D2095 isolate in *Galleria mellonella*, but higher long-term survival in minimal medium in aerobic conditions. Mucoid phenotype variation was also shown to be triggered by nutrient limitation, high antibiotic concentration, osmotic and oxidative stresses. Taken together, these results suggest that adaptation of Bcc within the CF lung environment involves genotypic and phenotypic variation among isolates, contributing to their fitness while maintaining their capacity for survival in this opportunistic human niche.
Zlosnik JEA, Hird T, Fraenkel M, Moreira LM, Henry DA, Speert DP. (2008). Journal of Clinical Microbiology 46:1470-1473 Silva IN, Ferreira AS, Becker JD, Zlosnik JEA, Speert DP, He J, Mil-Homens D, and Moreira LM. (2011). Microbiology 157:3124-3137.

OC3

Zebrafish as a model system of proteotoxic stress induced by mRNA mistranslation

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Cell homeostasis is sustained by an extensive network of protein quality control mechanisms. In order to elucidate how eukaryotic cells respond to proteome instability (proteotoxic stress) we are developing methodologies to destabilize protein structure on a proteome wide scale in a regulated manner. We developed approaches that circumvent the limitations of current methodologies that rely on overexpression of aggregation prone proteins or chemical stressors, whose secondary effects question their validity as model systems for studying cell degeneration processes. We have engineered transfer RNA (tRNAs) to misincorporate amino acids at low level into proteins on a proteome wide scale in zebrafish embryos. The mutant proteins misfold and are targeted to degradation or aggregation, triggering the proteotoxic stress response, which we are monitoring using biochemical, genetics and molecular assays. Some of the mutant tRNAs produced strong effects on embryo development and viability while others had minor effects only. Apoptosis, stress response and embryonic development pathways were deregulated, in particular, caspases 9, 8, 3 and 7, the unfolded protein response and ubiquitin proteasome pathways were activated. Moreover, mistranslation resulted in protein aggregation. In conclusion, misreading tRNAs are powerful tools to destabilize the proteome for investigating the biology of proteotoxic stress in vertebrates. Our approach targets the whole proteome with different mutations that specifically activate quality control mechanisms. This allows controlled proteome aggregation without compromising the function of specific proteins, mimicking protein aggregation processes that occur naturally during aging.
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OC4

Gene Expression and disease association analyses implicate EREG-AREG and NRG1 in susceptibility to Behçet's Disease

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Behçet's disease (BD) is a complex disease with genetic and environmental risk factors implicated in its etiology, however BD pathology is poor understood. The goal of our study was to molecularly dissect Behçet's disease pathways by mean of microarray technology in order to identify candidate genes to be tested for association with BD in a large cohort of BD cases and controls. Methods and Results: We first compared the gene expression profiles in PBMCs of 15 patients and 14 matched controls using Affymetrix HGU133 Plus 2.0 microarrays. Pathway analysis revealed that the Neuregulin signaling pathway was in the top represented pathways and that several genes of this pathway were among the top differentially expressed genes. EREG (fold-change=-2.39, P=2.99E-2), AREG (fold-change=-1.80, P=4.90E-2) and NRG1 (fold-change=-1.76, P=3.12E-2) were tested for association with BD in a cohort of 976 BD patients and 839 controls. A haplotype tagging SNP approach was used to select SNPs at the EREG-AREG locus and SNPs in NRG1 already found associated in one of the two GWAS for BD were also genotyped. We found a new association with BD of one SNP located downstream of EREG (rs2132065) and replicated three associations at NRG1 (rs4489285, rs383632, and rs1462891). Multidimensional Reduction Analysis indicated the existence of epistatic interactions between EREG and NRG1 variants. Conclusions: EREG, AREG and NRG1 are members of the epidermal growth factor (EGF) family and the EGF/ErbB signaling has been extensively demonstrated to control fundamental biological processes in mammalian cells. These association findings support a role for the EGF/ErbB signaling pathway in BD pathogenesis that warrants further investigation. EREG-AREG and NRG1 seem to modulate BD susceptibility through main effects and gene-gene interactions. This study highlights the importance of combining genetic and genomic approaches to dissect the genetic architecture of complex diseases.

OC5

Zebrafish as a model to unveil and study skeletal phenotypes related to human MEF2C mutations

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MEF2C is a transcription factor with important roles in differentiation and developmental processes. It is an important regulator of neurogenesis, muscle, lymphocyte, cardiovascular and endochondral bone development. Recently, an association between human MEF2C gene mutations and particular phenotypes affecting not only neurodevelopment and overall body development delay but also craniofacial formation has been shown. These data provide the first evidence linking human MEF2C with normal craniofacial and skeletal development. In another study aiming at determining the function of MEF2C in mice Neural Crest (NC) development, a conditional gene targeting approach to inactivate Mef2c in the NC was used. This inactivation results in lethality at birth due to upper airway obstruction. In addition, neonates also exhibited a posterior cleft of the palate, a constricted airway, and defective position of the tongue thus highlighting a role for MEF2C in mice craniofacial development. Altogether, the available information in mice and humans suggests a role MEF2C in craniofacial developmental. Zebrafish has been shown to be a good model to study genetic disorders, in particular those related to skeletal development and in addition it offers many technical advantages. Recent studies have shown that the zebrafish palate, or anterior neurocranium, is under a genetic control similar to the one controlling amniote palatal skeleton. It was also demonstrated that palatogenic cranial neural crest cells reside in homologous regions of the developing face compared with amniote species and that molecules regulating mouse palatogenesis are expressed in similar domains in zebrafish. These results suggest that the gene networks regulating palatogenesis may be conserved across vertebrate species, thus demonstrating the utility of zebrafish as a model to study palatogenesis and other aspects of craniofacial development. Zebrafish has two mef2c genes, mef2ca and mef2cb, located in chromosomes 10 and 5, respectively and different lines of mef2ca and mef2cb mutants are available. Phenotypes of homozygous and heterozygous fish from mef2cab1086 mutant line were analysed and found to share similarities with previously published phenotypes of MEF2C in null mice and humans thus proving additional evidence for the use of zebrafish as an alternative model to analyse in detail the role of mef2c in palate and craniofacial development. Our results using this model organism will be discussed.

P1

The role of MLPA in a Diagnostic Laboratory

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The MLPA (Multiplex Ligation-dependent Probe Amplification) is a semi-quantitative molecular technique that allows the detection of gains (duplications) and losses (deletions) in specific DNA sequences. Given its high sensitivity and specificity in the study of chromosomal imbalances, this technique is slowly replacing the FISH analysis. Nevertheless MLPA is unable to detect all the variations of ploidy and balanced chromosomal structural alterations, presenting low sensitivity to mosaicism in contrast to FISH which is applicable in this situations. However, both techniques can only be used in the case of known genetic alterations using specific probes, although the MLPA offers a better resolution and information. The objective of this work consists in the application of 5 MLPA panels for the study of patients with mental retardation (P036 and P070), microdeletion/microduplication syndromes (P245), autism (P343) and DiGeorge syndrome (P250). In the case of panels P036 and P070, useful in the analysis of subtelomeric regions, in terms of alterations (for a period of 3 years) a rate of 4,9% was detected. In panel P245 we have identified 1.3% of alterations and in panel P343 we have not found any chromosomal alterations. Finally, in panel P250 we have detected 2,7% of alterations relatively to the critical region of chromosome 22 (22q11.2). In the case of this panel, we have detected an atypical deletion of approximately 1Mb in 22q11.2 region, which is not identifiable by FISH. The parents were studied by MLPA and we have confirmed that this deletion was inherited from the father. This deletion is the first found to be inherited in region 22q11.2 in our laboratory. Nowadays the initial strategy in most of these studies is centralized in a CGH technology and MLPA is used in a complementary way for the confirmation of the detected alterations and parents studies. "MLPA design" is an alternative strategy in cases detected, by array with alterations in chromosomal regions not included in the commercially available MLPA panels. MLPA is an important technique in a diagnostic laboratory since it enables the detection of small submicroscopic alterations and the validation of results obtained by other technologies, namely the a CGH, in a short time, with high efficiency and low cost.

P2

The Controversial p.M34T Mutation in GJB2: Report on Three Portuguese patients with NSSHL

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Approximately 1 in 1000 individuals born with some kind of hearing impairment and single-gene defects account for over half of the cases of severe to profound sensorineural bilateral congenital childhood deafness. Mutations in GJB2 gene, coding for connexin-26 protein (Cx26, expressed in cochlea and forming subunits of gap junctions, are responsible for up to 50% of nonsyndromic sensorineural hearing loss (NSSHL). Two gross deletions, del(GJB6-D13S1830) and del(GJB6-D13S1854), involving GJB6 gene, which codes for connexin 30 (Cx30), have also been associated with NSHL in several cases. Both genes, GJB2 and GJB6, are localized to chromosome 13q11, corresponding to the DFNB1 locus. The pathogenicity of some GJB2 mutations is controversial, being p.M34T one of those variants, reported as a recessive allele by some authors, whereas other authors considered it a benign polymorphism. Insofar, the role of p.M34T is still not clarified, being necessary more genotype/phenotype studies of affected patients. The aim of the present study was to identify the genetic etiology of deafness in three Portuguese individuals, two sisters and one unrelated individual, presenting moderate to severe NSSHL. All individuals were evaluated in both ears by pure tone audiometry and blood samples were collected after written informed consent was signed. DNA extraction and PCR amplification of GJB2 coding region followed standard methodologies. PCR products were automatically sequenced in both directions and deletions in GJB6 gene were assessed by multiplex PCR. The two sisters were found to be compound heterozygous carrying two different GJB2 coding mutations, p.M34T and p.R184P. Their hearing mother is a p.M34T carrier and their hearing father is a p.R184P carrier. The other unrelated patient is a compound heterozygous harbouring two different mutations in GJB2 gene, p. M34T and p. I140S. All three patients carried no GJB6 deletion. Mutations p.R184P and p.I140S, aren't very common but are reported as recessive ones. So, they wouldn't explain by themselves the hearing impairment of two sisters and the other unrelated patient. In this way, the hearing loss of these Portuguese patients might be considered to be due, or likely due, to the GJB2 genotype [p. Met34Thr]+ [p. Arg184Pro] and [p. Met34Thr]+ [p. Iso140Ser], pointing to the pathogenic role of p.M34T, either as a recessive allele or as a polymorphism which increases the severity of another recessive monoallelic mutation.

P3

Effect of mucoid morphotype variation of *Burkholderia* isolates on gene expression, resistance to antimicrobial environments and virulence

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Burkholderia genus is a group of gram-negative bacteria that can be found in water, soil, in association with plants or as pathogens in humans and animals. Particularly, bacteria from *Burkholderia cepacia* complex (Bcc) are well-known opportunistic pathogens infecting cystic fibrosis (CF) or immunocompromised patients. Since CF is caused by mutations in a chloride ion channel protein, thus affecting mucus clearance in lungs, infection with multi-resistant Bcc bacteria leads to chronic lung inflammation and in some cases patients develop septicemia. Bcc pathogenicity is related to several virulence factors, one of them being the exopolysaccharide (EPS) cepacian which is also a common feature in the genus *Burkholderia* conferring these bacteria a mucoid phenotype. Previous studies reported that mucoid-to-nonmucoid conversion was shown to occur in Bcc long-term infections of CF patient airways. Here we show that mucoid-to-nonmucoid phenotype variation occurs in clinical and in environmental Bcc and non-Bcc *Burkholderia* strains in response to nutrient starvation and to ciprofloxacin. Expression profiling of *B. multivorans* D2095 and ATCC 17616 isolates vs. non-mucoid D2095nm and ATCC 17616nm variants obtained under nutrient starvation conditions was made using a custom microarray. The results have shown the altered expression of genes from bceI and bceII clusters encoding proteins involved in EPS biosynthesis, genes encoding fimbrial proteins and involved in flagellar biosynthesis and chemotaxis. Phenotypic characterization of mucoid isolates and nonmucoid variants obtained under nutrient starvation or in the presence of ciprofloxacin was also performed. In general, nonmucoid strains showed higher siderophore production, higher antibiotic and SDS susceptibility and attenuated virulence in *Galleria mellonella* model of infection. Swimming and swarming motility showed differences between mucoid and nonmucoid strains but no correlation with the EPS production was established. Lipopolysaccharide pattern and resistance to oxidative and osmotic stresses showed no significant differences between mucoid and nonmucoid strains. Understanding morphotype variation signals and mechanisms represents a key area for our future studies.

P4

Molecular Diagnosis of Autosomal Dominant Polycystic Kidney Disease: Mutation Detection in PKD1 Gene

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Autosomal Dominant Polycystic Kidney Disease (ADPKD) is a common hereditary and monogenic disorder (1/600-1/1000) that results in renal cysts development that increases in number and size as the person gets older, often leading to end-stage renal disease. PKD1 is a large (~47kb) and complex gene that comprises 46 exons, codifies the protein polycystin 1 and accounts for 85% of the identified cases of ADPKD. Currently, the full sequencing of PKD1 allows, with high sensibility, the detection of variations along the gene and this may be used in certain clinical situations where imaging cannot provide a definitive clinical diagnosis. However, the screening of mutation is often inconclusive because of multiple homologous copies of this gene on chromosome 16. Besides that, more than 800 PKD1 variants have been described, which complicate the genetic diagnosis. The research presented here is focused on the development of a genetic diagnostic test for ADPKD through the complete PKD1 coding region sequencing, using Sanger technology, that allows identifying potential pathogenic mutations related to the development of this disorder. The total coding region of PKD1 was amplified as thirteen distinct PCR fragments with sizes between, approximately, 500 and 5000bp. Using BigDye® Terminator V3.1 technology sequencing, all the fragments were successfully sequenced and neutral polymorphisms in control samples were detected. This research demonstrated the potential of automated Sanger sequencing for diagnosis of genetic diseases. Although further validation using samples from ADPKD patients is still needed, we concluded that this approach can lead to an effective molecular genetic diagnosis. Extension of this work to the next generation sequencing platform Ion Torrent is currently in progress in our laboratory.

P5

Development and implementation of a new protocol for screening of EGFR Mutations from FFPE samples of Non-Small Cell Lung Cancer Patients

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Background: Lung cancer is the leading cause of cancer-related death accounting for one third of all deaths from cancer worldwide. Somatic mutations in the epidermal growth factor receptor (EGFR) have been described in patients with advanced non-small cell lung cancer, located in the exons 18-21 of the tyrosine kinase (TK) domain. Exon 19 deletions and the exon 21 L858R mutations account for 90% of all EGFR mutations, while the remaining 10% are harbored in exon 18 and exon 20. These mutations induce constitutive activation of the tyrosine kinase (TK) domain of the receptor and confer dramatic sensitivity to TK inhibitors (TKIs) gefitinib and erlotinib, while the latter has shown to be resistance related as it renders the receptor about 100-fold less sensitive to EGFR-TKIs. Screening of EGFR mutations, both for selecting patients for treatment with TKIs and for detecting the resistance mutation, is thus extremely important for treatment decision. Purpose: The aim of this study is to develop a new laboratory routine for the screening of EGFR mutations using direct automated Sanger sequencing and fragment analysis from formalin-fixed and paraffin embedded (FFPE) samples. Materials and Methods: Three different type of samples were used for the development and validation of this work: a) blood sample on FTA Indicating Micro Card for PCR and multiplex PCR DNA amplification ; b) gDNA from MCF-10a cell line containing the exon 19 E746-A750 deletion for limit of detection determination associated with Sanger technique and fragment analysis by capillary electrophoresis; c) FFPE samples from 10 different patients for EGFR mutation screening by automated sequencing and fragment analysis. Results: We have successfully developed a PCR protocol for EGFR mutation detection through direct Sanger sequencing and fragment analysis, and screened all 10 FFPE samples for exon 19 and 21, and 5 out of 10 for exons 18 and 20. We also managed to detect deletion mutation in gDNA from MCF-10a cell line in samples containing 50% down to 1% mutation. Multiplex PCR procedure is currently being optimized. Conclusion: Our protocol for the screening of EGFR mutations using Sanger sequencing and fragment analysis has shown to be effective, though optimization is still required in sequencing samples amplified by multiplex PCR.

P6

Analysis of FTO (rs9939609) and PPARG (rs1801282) involvement in obesity in the Portuguese population

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Obesity is a medical condition developed from the excessive accumulation of fat mass that may increase health problems and decreased life expectancy, which may result from the combination of different genetic, environment and lifestyle factors. There are only scattered reports on the genomic involvement in obesity in the Portuguese population. This study aimed to describe two related obesity SNPs, i.e. Fat mass and obesity associated-gene (FTO) rs9939609 and Peroxisome proliferator-activated receptor gamma (PPARG) rs1801282 and their risk to obesity in the Portuguese population. Method: A total of 194 Portuguese female Caucasian in premenopause aged between 18-50 years old participated in this study to assesses the risk of rs9939609 (FTO) and rs1801282 (PPARG) to obesity. Ninety-five subjects were classified as case (BMI≥30 Kg/m2) and ninety-nine as control (BMI between 18.5 and 24.9 Kg/m2). Polymorphism characterisation was performed via direct Sequencing using Big Dye V3.1 technology. The association of the SNPs with obesity as determined by odd ratios (ORs) calculation with 95% of confidence interval (CI) Results: Significant differences in BMI between control and case group for FTO rs9939609 (P0.05). Conclusion: This is the first study in the Portuguese population showing the association between FTO and PPARG gene and obesity. These results are important for the clinical management of obesity.

P7

Bisphenol A modifies *C. elegans* developmental patterns and induces genetic alterations in the progeny

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Bisphenol A (BPA) is a widely produced chemical used in a variety of consumer products including plastic food containers and to which human exposure is considered generalized (Myers et al. 2009, Environ Health Perspect. 117:309). BPA toxicity is still highly controversial although numerous effects have been described (Rubin 2011, J Steroid Biochem Mol Biol. 127:27). In the model organism *Caenorhabditis elegans* it was recently shown that BPA disrupts double-strand break repair (DSBR) mechanism during meiosis (Allard & Colaiácovo 2010, PNAS 107:20405). Here we compared the effects of BPA exposure in the wild type strain N2 and fan-1 (a mutant that shows hypersensitivity to DNA crosslinking agents). A life cycle staging bioassay was performed with synchronized cultures to assess BPA influence in worm development. Synchronization was achieved by egg release using standard bleaching method. Eggs were plated in control medium or with medium supplemented with 0,5mM or 1mM of BPA and allowed to develop for 72h at 20ºC. In control medium 90% of worms reach L4/adult stage and no early developmental stages (L1, L2) are observed. Conversely, in the presence of BPA an obvious desynchronization was observed characterized by the presence worms in every developmental stage (L1, L2, L3, L4 and adult). This shows that BPA exposure alters the highly stable developmental pattern both in N2 and fan-1. Additionally, we evaluated the induction of genetic alterations in the progeny of BPA exposed individuals. For this purpose worms developed in the presence of BPA were allowed to lay eggs in BPA free medium and descendants were maintained until L4/adult stage. Genetic profiles were compared between progenitors and descendants (at least 10 per progenitor) using random amplified polymorphic DNA (RAPD) and single worm PCR. RAPD banding profiles were established for two distinct primers using 20 individuals grown in control conditions. The results obtained show that the banding patterns of the exposed progenitors are identical to that of controls. However, all the individuals analyzed in the progeny of exposed individuals showed altered banding patterns in relation to progenitors. These alterations are characterized by the loss of bands that are always present in controls as well as by the occurrence of new bands never observed in controls. This data clearly shows that BPA exposure induces DNA modifications in germ cells that are transmitted to the next generation.

P8

Burkholderia cenocepacia pathogenesis: unraveling the virulence functions of trimeric autotransporter adhesins

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Burkholderia cepacia complex bacteria have emerged as highly problematic opportunistic pathogens in patients with Cystic Fibrosis (CF). To initiate infection, bacteria must be able to colonize the respiratory epithelium. This step is mediated by several adhesins, such as trimeric autotransporter adhesins (TAAs). These homotrimeric proteins are involved in various biological traits of pathogenic bacteria. We had previous identified in the genome of the epidemic *B. cenocepacia* J2315, a novel cluster of genes harboring three TAAs (BCAM0219, BCAM0223 and BCAM0224). In this thesis we performed the functional characterization of these TAAs, determining their roles in pathogen-host interactions and virulence. The expression of these genes occurred preferentially for cells grown under oxygen-limited conditions, osmotic and oxidative stress. We also proved their involvement in binding to extracellular matrix proteins, hemagglutination, biofilm formation, serum resistance, virulence using *Galleria mellonella* as model host and adhesion to and invasion of human lung epithelial cell line 16HBE14o- and CFBE41o- (non-CF and CF phenotype, respectively). Altogether our results demonstrate that these TAAs are important multifunctional virulence factors of *B. cenocepacia*, playing different roles in pathogenicity.

P9

Zebrafish: a good model to study XPG pathologies?

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Transcription factor II-H (TFIIH) is one of several general transcription factors that make up the RNA polymerase II pre-initiation complex and it also plays a very important role on nucleotide excision repair (NER) mechanism as well as in cell cycle regulation. Generally it is considered that TFIIH is made up of 10 subunits which can be regarded as forming two subcomplexes, namely the core of TFIIH, formed by XPD, XPB, p62, p52, p44, p34 and TTDA, and the cyclin activating kinase (CAK) complex, which contain CDK7, MAT1 and cyclin H. Additionally, the XPG protein may be considered the eleventh subunit of TFIIH since it strongly interacts with this complex, helping in its stabilization and participating in transcription and DNA repair. Recently, our laboratory has shown that the 10 accepted members of TFIIH already described are well conserved between human and zebrafish. In contrast, for XPG, an endonuclease that cuts a DNA lesion at its 3' end during NER, nothing is known concerning its conservation and role in zebrafish. Mutations in the XPG gene are primarily associated with NER disorders like Xeroderma Pigmentosum (XP) and XP-Cockayne Syndrome combined features, but there are also evidences that single nucleotide polymorphisms in XPG are related with an increased risk of certain types of cancer. The main objective of this study is to investigate if XPG gene and protein structures were maintained throughout evolution in order to provide additional evidence towards validation of zebrafish as an alternative model to analyse this gene and its function as part of the TFIIH complex . In this study we will therefore provide information on zebrafish XPG including its gene and protein structures, types of transcripts, presence of alternative spliced variants and their tissue distribution, as well as data on its overall conservation between humans and zebrafish.

P10

Modulation of HIV-2 infectivity by APOBEC3 family

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Infectivity of Human immunodeficiency virus type 1 and 2 require a virus encoded gene product, Vif (Virion infectivity factor). Vif1 counteracts an anti-retroviral cellular factor expressed in non-permissive cells, named APOBEC3G. Vif1 induces APOBEC3G degradation preventing the presence of APOBEC3 in viral particles which is related with higher levels of infectivity of HIV-1. APOBEC3G is one of the members of the APOBEC3 gene family coding for cellular cytidine deaminases which all seem to be able to inhibit HIV-1.
Vif1 can be functionally replaced by Vif2 but their identity is very small (25%). Few studies were performed to evaluate differences in the ability of each member of APOBEC3 family to inhibit HIV-2 production and infectivity which could explain the lower infectivity of HIV-2 compared with HIV-1. For instance, we have showed previously that APOBEC3G is less efficient to inhibit HIV-2 compared with HIV-1.
Our aim is to characterize inhibition levels of HIV-2 infectivity due to interactions between each member of APOBEC3 family and Vif2. We will perform degradation and single-cycle infectivity assays in the presence of different APOBEC3 proteins, using HIV-2 vif-deficient and wild-type.
Preliminary results show that Vif2 seems to be very efficient to degrade APOBEC3C and 3G. However, APOBEC3F seems to be the most efficient APOBEC3 to inhibit HIV2 infectivity.

Symposium 2
Gene Regulation and Signaling Pathways

Coordinators: Cecília Rodrigues (iMed, FF, UL) and Manuel Santos (CESAM, UA)

OC6

Involvement of the BceF tyrosine kinase in stress response, biofilm formation and virulence in *Burkholderia cepacia* IST408 clinical isolate

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BceF protein from *B. cepacia* IST408 belongs to the bacterial tyrosine kinase family of proteins known to regulate polysaccharide biosynthesis, DNA metabolism and stress responses. Despite being involved in the exopolysaccharide cepacian biosynthesis [1], it was our aim to determine other cell targets that may be dependent on BceF activity. To achieve that, we performed an expression profile experiment between the bceF mutant and the wild-type *B. cepacia* IST408 resulting in 630 genes differentially expressed. Genes with decreased expression in the bceF mutant were related to stress response, motility, cell adhesion and outer membrane composition. Genes with increased expression in the bceF mutant were related to intracellular signalling, type VI secretion and iron metabolism. Accordingly to these results we observed a decreased survival rate of the bceF mutant exposed at 50ºC when compared to the wild-type strain, reduced swimming motility, and having increased intracellular levels of c-di-GMP at 24h of growth. Furthermore, bceF mutant is unable to form mature biofilms, with the parental strain forming well differentiated microcolonies (beginning of mushroom-like structures), while the mutant presented small cell aggregates, without covering the complete chamber surface. The relevance of BceF in *B. cepacia* virulence was confirmed by decreased mortality of *Galleria mellonella* larvae and by mutant's inability to adhere and invade human lung epithelial cells and to disrupt the epithelium tight-junctions, which enables bacteria to reach paracellularly the basolateral surface. These findings confirm the involvement of BceF in many cellular processes and now the challenge is to find its precise phosphorylation targets within the cell.

OC7

Epigenetic Regulation of microRNAs Expression in Breast Cancer

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MicroRNAs (miRs) are a class of non-coding RNAs that regulate gene expression by targeting several mRNAs. Emerging evidence indicates that deregulated miRs are involved in the development of cancer, including breast cancer (BC), the prevailing cancer among women in industrialized countries. MiRs that inhibit translation of proto-oncogenes are considered tumour suppressor miRs and are down-regulated in cancer, while other miRs are up-regulated in cancer and may act as oncogenic miRs by down-regulating tumour suppressor genes. The regulation of miRs expression is an emerging field in cancer, in particular their epigenetic regulation through promoter CpG island methylation. In order to study the promoter CpG island methylation of miRs in BC we selected a set of several CpG islands in promoter region of various miRs. In a first approach, we measured by qPCR 95 miRs in the MCF-7 cell line before and after a challenge with the demethylating agent 5-aza-2'-deoxycytidine (DAC). We then selected a set of miRs which presented CpG islands in the promoter region (http://genome.ucsc.edu). These sets of CpG islands were then evaluated in MCF-10A (non-tumourigenic), MCF-7 (tumourigenic) and MDA-MB-231 (tumourigenic metastatic) cell lines by Methylation Specific PCR (MSP). Among the miRs analysed, the promoter region of let-7a-3 is totally methylated in MDA-MB-231, almost totally methylated in MCF-7 and partially methylated in MCF-10A. The miR-199b promoter region is totally unmethylated in the non-tumourigenic MCF-10A cell line and partially methylated in the tumorigenic cell lines. This suggests that these two miRs are prone to methylation during breast tumourigenesis and are important regulatory factors in mammary healthy cells. Let-7a-3 promoter hypermethylation was already found to be associated with epithelial ovarian cancer and BC since its low methylation was related with high expression of insulin-like growth factor-II. According to DIANA-mirPath, this miR seems to play an important role in other signaling pathways, such as the MAPK signaling pathway, since it can putatively regulate 19 genes involved in this pathway. Methylation of the miR-199b promoter hasn't been associated with breast cancer. MiR-199b seems to have an important role in MAPK signaling pathway and ErbB signaling pathway. Our next goal is to confirm these miRs expression in tumour samples and evaluate the importance of these miRs in the mentioned pathways.

OC8

Gene silencing of the negative regulator of inorganic sulfur assimilation SconC affects virulence of the fungal pathogen *Paracoccidioides brasiliensis*

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Mycelium-to-yeast transition of the fungus *Paracoccidioides brasiliensis* in human host is a critical step for the establishment of paracoccidioidomycosis, a systemic mycosis endemic to Latin America. Up to now the master regulators mediating morphogenesis, virulence, and the mycelium-to-yeast transition are still elusive. Besides the specific temperature requisite, a known feature affecting *P. brasiliensis* mycelium-to-yeast transition is the auxoptrophic nature of the pathogenic yeast form for sulfur aminoacids. Considering the importance of PbSconC as a negative regulator of the inorganic sulfur assimilation pathway and given its increased expression in the pathogenic yeast form, we downregulated its expression by gene silencing to better understand this phenomenon and its implications on *P. brasiliensis* virulence. In this work we were able to promote mycelium-to-yeast transition in the PbSconC knock-down mutants in medium without organic sulfur compounds, contrarily to wild-type strains that are arrested in the hyphal form under such conditions. Nonetheless, SconC knock-down cells were not able to sustain yeast form growth. Flow cytometry evaluation showed that knock-down strains have higher basal oxidative stress, using dihydrorhodamine and dihydroethidium staining analysis. Overall, these results raise the possibility that SconC downregulation impairs the redox balance. To assess the role of this gene on *P. brasiliensis* virulence, C57BL.6 males were intravenous infected with 1x106 cells/mL. Our results show that the SconC *P. brasiliensis* mutants are less virulent. Overall, the data herein presented gives new insights on the molecular biology of fungal dimorphism, and on the identification of new virulence determinants.

OC9

Tissue specific requirements of *Drosophila* NTC complex in pre-mRNA splicing and transcriptional elongation

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The NineTeen complex (NTC) is an integral component of the active spliceosome and is essential for both steps of intron removal during pre-mRNA splicing. The NTC is also important for transcriptional elongation and RNApolII processivity, which suggests a role of this complex in the functional crosstalk between transcription and pre-mRNA splicing. We isolated a maternal mutant defective for *Drosophila* blastoderm cellularization. This mutant was named fandango, and deficiency mapping and cloning showed it was allelic to a conserved core-subunit of the NTC complex. fandango mutant embryos showed a dramatic reduction in Fandango protein levels and significant splicing defects of early zygotic but not maternal pre-mRNAs. fandango mutant embryos also showed defects in transcriptional elongation of early zygotic genes. This suggested Fandango was rate limiting for gene expression during early embryogenesis but not during female germ-line development. We hypothesize that during early embryogenesis, the fast syncytial nuclear divisions impose significant constraints to gene expression, which makes Fandango function particularly rate-limiting during this stage of development. Consistent with this hypothesis, *Drosophila* early zygotic genes are usually small and intronless.

OC10

Functional analysis of mating genes of the dimorphic fungal pathogen *Paracoccidioides brasiliensis*

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Paracoccidioidomycosis the most prevalent systematic mycosis in Latin America, caused by the thermodimorphic fungus *P. brasiliensis* with unknown sexual reproduction system. The genomes of three sequenced *P. brasiliensis* strains (Pb01, Pb03 and Pb18) encode highly similar homologues of mating-related genes from other Ascomycete fungi: the MAT1-1/MAT1-2 idiomorphs, pheromone receptors and mating signaling pathway components. A recent study showed a 1:1 distribution of MAT1-1 and MAT1-2 idiomorphs in *P. brasiliensis* isolates from various sources. These results strongly support the occurrence of sexual reproduction within the Paracoccidioides genus. We observed low expression levels of MAT1-1, MAT1-2, alpha-factor (Pbα) and the receptors for alpha-pheromone (PreB) and a-pheromone (PreA) in both mycelia and yeast form, indicating that such can contribute to the eventual low fertility. Functionality of *P. brasiliensis* mating genes was further assessed by heterologous expression in *S. cerevisiae* mating gene null mutants. Our data reveal that exposure of *S. cerevisiae* strains expressing PreB to both *P. brasiliensis* culture extract and synthetic Pbα pheromone induces shmoo formation and growth arrest evaluated by halo-assays, decrease of specific growth rate and G0/G1 cell cycle arrest at. This demonstrates pheromone signaling activation by *the P. brasiliensis* alpha-pheromone and pheromone-receptor recognition. Heterologous expression of Pbα and PreB in *S. cerevisiae* null mutant strains of opposite mating types allowed restoring mating fertility. In conclusion, our results indicate novel evidence for the existence of a functional mating signaling system in *P. brasiliensis*. Additional experiments are currently in progress to address the molecular details of mating signaling in this fungus and to identify environmental conditions for mating.

OC11

Bacteria can silence genes too: RNase III and small RNAs seek and destroy outer membrane transcripts

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Bacteria resort to all kind of intricate strategies to cope with changes in the outside world. For instance, the expression of the main outer membrane proteins is regulated by small RNAs (sRNAs), which do not encode for proteins. These sRNAs are in turn regulated by the activity of RNA-degrading enzymes (RNases) (Silva et al. 2011). This story revolves around MicA, a sRNA conserved among many enterobacteria that has been reported to be involved in *Salmonella* virulence mechanisms. Its main targets are the outer membrane protein OmpA and the maltoporine LamB. To understand the regulation by this sRNAs it is fundamental to study its turnover, which may impact on the regulatory capacity of MicA. The aim of this work is to study the degradation of MicA. We have purified the RNase E and RNase III endoribonucleases of *Salmonella* that were previously shown to have an impact in MicA stability, and have performed in vitro activity assays to clarify their role on the decay of this sRNA. We have also analyzed the stability of MicA in the presence of its known mRNA targets to investigate whether there is a coupled MicA-target degradation. RNase III, which is not able to cleave free MicA, is shown to cleave very efficiently the sRNA when it is coupled with ompA or lamB mRNAs (Viegas SC, Silva IJ et al. 2011). This work uncovers the regulation of MicA sRNA pointing to a gene silencing mechanism similar to that of higher organisms, a process known as RNA interference or gene silencing. The fact that, in *Salmonella*, MicA is cleaved by RNase III in a target-dependent fashion, with the concomitant decay of the mRNA target, strengthens the RNase III role in the regulation of bacterial gene expression.
Silva I.J., Saramago M,. Dressaire C., Domingues S,. Viegas S. C., and Arraiano C. M. 2011. Importance and key events of prokaryotic RNA decay: the ultimate fate of an RNA molecule. WIRES. 2: 818-836 Viegas S. C, Silva I.J., Saramago M., Domingues S., and Arraiano C. M. 2011. Regulation of the small regulatory RNA MicA by ribonuclease III: a target-dependent pathway. Nucleic Acids Res. 39(7):2918-30.

OC12

Morphodynamics of spore differentiation in relation to pathogenesis in *Clostridium difficile*

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Clostridium difficile is a strict anaerobic spore-forming bacterium and the major causative agent of nosocomial diseases associated to antibiotic therapy in adults. Spores produced by *C. difficile* are highly resistant, infective and are the primary cause of transmission in health care institutions. Despite the central importance of spores in the pathogenesis of *C. difficile*, a detailed description of the cell differentiation process leading to spore formation in this organism is lacking. Evidence suggests that the main morphological stages of sporulation are conserved in the Clostridia. Moreover, the four cell type-specific RNA polymerase sigma factors that govern developmental gene expression in the well-studied model organism *Bacillus subtilis* are present in the *C. difficile* genome. In *B. subtilis*, σF in the forespore, and σE in the mother cell, control early stages of development, and are replaced by σG and σK, respectively, which control the final stages of development. Using a recently developed genetic system based on the use of type II introns (ClosTron), we have inactivated sigF, sigE, sigG and sigK of *C. difficile*. Disruption of sigF, sigE and sigG abolishes spore formation and their function largely conforms the *B. subtilis* paradigm. In contrast we found that σK is not essential for sporulation. Rather, σK appears to be specifically required for the release of the spore from the mother cell into the surrounding medium. Because the sigF mutation confers the earliest specific block in the sporulation pathway the mutant was used to assess the requirement for sporulation for colonization. We found that cells of a sigF mutant colonized human flora-associated mice with the efficiency of wild type *C. difficile* cells. While not ruling out a role in the process, our results show that sporulation is not an absolute requirement for colonization. Nevertheless, we show that a gene controlled by σK and strongly induced in vivo in an axenic mouse model, is required for efficient colonization. We show that this gene is also a key determinant of the assembly of the spore surface layers. These results provide the first direct link between the spore surface and the colonization process.

P11

Gender related differences in the rat choroid plexus transcriptome

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The choroid plexus (CP) is a vascular fringe structure that projects into the ventricules of the brain, with a pivotal role in the protection of the central nervous system (CNS). The best recognized function of the CP is cerebrospinal fluid (CSF) production, which is responsible for intracranial volume adjustment, buffering of brain extracellular fluid ions and other solutes, and drainage and transportation of peptides and neurotransmitters within the CNS. A large number of neuropeptides, growth factors and cytokines have been identified in the CP, which are differentially regulated by the hormonal background. However, little is known about the complete CP transcriptome and particularly, differences between the male and female transcriptomes have never been reported. Therefore, a microarray analysis comparing male and female rat CP transcriptomes was performed for a better understanding of gender related differences in CP functions in health and disease. Significant differences between male and female rats were found in the number of genes related with neurodegenerative diseases as Alzheimer, Parkinson and Huntington's diseases, oxidative phosphorylation, and apoptosis. These data suggest that gender can have an impact on the expression of genes associated with neurodegenerative diseases and essential cellular pathways in CP, highlighting the relevance of gender related differences in the functions of this tissue.

P12

Instability of mRNA expression signatures of drug transporters over time in chronic myeloid leukemia patients sensitive and resistant to imatinib

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Despite the success of Imatinib (IM) in the treatment of chronic myeloid leukemia (CML), about 30% of patients are resistant to therapy, most due to unclear causes. To profile the expression signatures of drug transporters through IM therapy and correlate them with resistance, we quantified mRNA expression levels of SLC22A12, ABCB1, ABCC1, ABCG2 and MVP in consecutive samples of CML patients who were either responsive or resistant to IM. Additionally we identified and quantified BCR-ABL1 transcript variants and analyzed 1236T>C ABCB1 and 480G>C SLC22A1 polymorphisms. No relation between the type of BCR-ABL1 transcript or ABCB1 or SLC22A1 genotype and response to treatment was found. However, the studied genes had higher expression levels in follow-up than in diagnosis samples, indicating a possible induction in expression. IM-sensitive patients presented significantly higher values of SLC22A1 expression, suggesting higher drug influx. Irrespectively of the response to treatment, gene expression values over time were correlated for most genes. However, while responding patients showed stable expression signatures in consecutive samples, there was considerable variation in IM-resistant patients, indicating that single point sampling expression signatures are not reliable to predict clinical outcomes or prognostic features in these patients.

P13

The food flavouring myristicin from nutmeg induces apoptosis and modulates gene expression in leukaemia K562 cells

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Spice-derived phytochemicals have been widely studied by several authors for their potential in the prevention and treatment of cancer. They can induce apoptosis, suppress proliferation of tumour cells, inhibit invasion and angiogenesis, mediating their effect through multiple targets. Extensive research within the last half a century has revealed that cancer is caused by deregulation of as many as 500 different gene products. Most natural products target multiple gene products and thus are ideally suited for prevention and treatment of various chronic diseases, including cancer. Alkenylbenzenes such as estragole, eugenol and myristicin are present as natural constituents of various spices and food flavours. Myristicin is found in nutmeg, basil, anise, cinnamon, clove, and their essential oils. It is also used in traditional medicine to treat rheumatism, cholera, psychosis, stomach cramps, nausea, diarrhea, and anxiety. The average consumption is about 9 mg/day from consumption of spices and approximately 4 mg/day from their use as essential oils. In our previous work we showed that myristicin has apoptotic activity without being genotoxic. Here we present an overall study in chronic myeloid leukaemia cells (K562) mainly focusing on gene expression and apoptosis. Alterations in gene expression were analyzed in 84 DNA damage-response genes (RT-PCR Arrays) after exposure to 100ÂμM myristicin for a 6 hour-period. These genes are involved in DNA Repair, apoptosis and cell cycle pathways. Apoptosis was evaluated using concentrations from 10 to 500ÂμM and exposure periods from 6 to 48 hours, by western blot (protein expression), caspases-3 detection, mitochondrial membrane potential dissipation (JC-1) and TUNEL assay. Gene expression analysis showed a pleiotropic effect, giving rise to a systematic deregulation of genes involved in cell cycle, DNA damage response, and apoptosis pathways. Features of apoptosis started at 100ÂμM exposure to myristicin, with mitochondrial transmembrane potential dissipation, cytochrome c release, caspases-3 activation and internucleossomal DNA fragmentation. Overall, myristicin presented apoptotic activity mediated by activation of caspases and exerts a pleiotropic effect influencing expression of the DNA-damage response genes, suggesting that this spice-derived phytochemical can be considered for further studies as a potential chemotherapeutic agent.

P14

E-cadherin expression in benign and malignant canine melanocytic tumours

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Cell-cell communication is necessary for the crosstalk between cells that constitute multicellular organisms. Adhesion molecules have been assigned an important role in melanocytic tumor progression. E-cadherin is a calcium-dependent cell-cell adhesion molecule expressed by melanocytes and responsible for their adhesion to keratinocyte. The aim of this study was to investigate the immunoexpression of E-cadherin in canine cutaneous melanocytic tumors and its correlation with tumor diagnosis. The expression of E-cadherin was evaluated by immunohistochemistry in 30 canine cutaneous melanocytic tumors, and scored in terms of labeling location (membranous, cytoplasmic and heterogeneous), extension and intensity. Only 2 tumours (malignant melanomas) were negative for E-cadherin. Membranous E-cadherin staining was generally absent in dermal nests of melanomas, whereas it was present in a high proportion of benign lesions (melanocytomas). E-cadherin immunoreactivity in malignant melanomas displayed a heterogeneous or diffuse cytoplasmic staining. However, the differences were not statistically significant (p=0,055). Both benign and malignant tumours had a diffuse moderate or strong reaction. E-cadherin staining was detected in all benign and almost all malignant lesions, however with a different pattern in labeling localization. These findings indicate that changes in the cellular expression localization of E-cadherin could occur in melanocytic lesions and may reflect different stages in their progression.

P15

Functional characterization of two genetic determinants involved in cell wall damage survival

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Cell wall damage invariably leads to bacterial cell death, but the physiological steps between these two events are not understood. The transcriptional analysis of two different murF mutants sharing the same cell wall defect - an insertion mutant (F9) and a conditional mutant (COLspacmurF) - showed very distinct expression profiles. One of the most significant differences was the high up-regulation (over 200 fold) in the insertional mutant, of two small ORFs, automatically annotated in the databases as conserved hypothetical proteins, present exclusively in staphylococcal species. No changes were observed in the expression of the two ORFs in the conditional mutant. The insertion mutant is completely adapted to the inflicted cell wall damage, while the conditional mutant is undergoing an adaptation process every time the inducer is removed. These observations suggested that these unknown ORFs play an important role in the adaptation of the cell to an impaired wall, ensuring survival. The C-terminal of one of the proteins shares 48% identity with a chemotaxis sensory transducer and the other shows 40% identity with a diguanylate cyclase. According to the TIGR-CMR database, the predicted proteins encoded by these two ORFs for *S. aureus* strain COL have expected molecular weights of 8.2 KDa and 7.6 KDa. However, for *S. aureus* strains NEWMAN and NCTC8325, according to the same database, a single protein of 12.7 KDa is predicted, as a different start codon is considered. Furthermore, expression of these proteins is not described, even in wide proteomic studies. To confirm that the ORFs are translated into protein products, crude protein extracts of strain COL and F9 were separated by SDS-PAGE and low-molecular weight bands which were present only in F9 were analysed by mass spectrometry. The identification of the two proteins confirmed that each ORF is translated into a different protein. Currently, both proteins are being over-expressed as His-tag fusions proteins in order to purify them to assess their role as well as to determine possible interactions between them. A knock-out mutant providing information about the essentiality of the ORFs and allowing to verify affected pathways as well as changed phenotypes in absence of the ORFs is also under construction.

P16

Development of a drug screening platform of p53/Mdm-2 protein-protein interaction

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The p53 protein is an ubiquitous transcription factor involved in the regulation of several cellular processes, such as cell cycle arrest, senescence and apoptosis. Therefore, mutation and deregulation of p53 is frequently associated with several pathologies, such as cancer and neurodegenerative diseases. Recently, the modulation of the interaction between p53 and its main negative regulator Mdm-2 has been highlighted as a promising therapy. Several compounds, such as nutlins were designed to disrupt this interaction. On the other hand, bimolecular fluorescence complementation (BiFC) analysis enables direct visualization of protein interactions in living cells. We aimed to develop a simple and robust platform to screen p53/Mdm-2 complex modulators and assess their effectiveness and therapeutic applicability. To evaluate p53/Mdm-2 interaction, we generated a Venus-based BiFC assay by constructing several eukaryotic expression plasmids containing p53 and Mdm-2 fused to two non-fluorescent halves of the Venus fluorescent protein (V1 or V2). The well-described interaction between p53 and Mdm-2 would bring the two non-fluorescent halves together, leading to the reconstitution of the functional fluorophore. Our results confirm the expression of recombinant p53 and Mdm-2 by western blot. In addition, we used human hepatoma and colorectal carcinoma cell lines co-transfected with different BiFC pairs to evaluate complex formation by fluorescent microscopy and flow cytometry analysis. The increased fluorescence production revealed that the best BiFC pairs were V1-p53 along with Mdm-2-V2 and V1-Mdm-2 with p53-V2, as compared to controls. Further studies are needed to optimize the sensibility and specificity of the assay. p53/Mdm-2 BiFC-based strategies may be used with, or instead of, traditional target-based drug discovery strategies to develop and identify novel, highly effective, and selective drug candidates, namely anti-tumor or anti-apoptotic compounds.

P17

ERK5 and MEK5 are overexpressed in colon adenomas and adenocarcinomas

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ERK5 and its direct activator, MEK5, are overexpressed in breast and prostate cancer in association with increased proliferation and chemoresistance, correlating with poorer disease prognosis. To date, there is no data on ERK5 or MEK5 expression in colon cancer (CC). However, ERK5 is directly regulated by miR-143, which is typically downregulated in CC. In addition, we have shown that miR-143 overexpression reduces viability and sensitizes CC cells to 5-FU, while reducing ERK5 and NF-κB protein expression. Further, silencing ERK5 via siRNA also increased CC cell apoptosis, as well as 5-FU-induced apoptosis, highlighting the potential relevance of ERK5 in CC. In this study, we evaluated the steady-state expression levels of ERK5 and MEK5 in a large set of 287 well-defined human colon samples, including normal colonic mucosa, tubulovillous adenomas, adenocarcinomas with proficient DNA mismatch repair (pMMR) and sporadic adenocarcinomas with defective DNA mismatch repair (dMMR). We also evaluated the steady-state protein expression of other signaling effectors of ERK5 signaling network, NF-κB and its inhibitor IκB, and p-AKT and AKT. Total proteins were collected from Trizol-chloroform fractions stored for 2 years at -80ºC, and steady-state protein expression was evaluated by immunoblotting. Our results clearly show that ERK5 and MEK5 are overexpressed in colon adenomas ($p < 0.01$) and carcinomas ($p < 0.05$), suggesting that overexpression, and possibly overactivation of ERK5 signaling occurs in CC, and may be relevant to disease onset and progression. Our results also suggest NF-κB is overexpressed and overactivated in colon adenomas, and carcinomas, compared to normal colon ($p < 0.05$). In turn, p-AKT and AKT displayed increased steady-state levels in adenomas and carcinomas. The activation ratio p-AKT/AKT significantly increased in adenomas compared to normal colonic mucosa ($p < 0.05$), and remained unchanged in carcinomas. In conclusion, our data provides evidence that ERK5 may be a novel and relevant therapeutic target in CC, deserving further investigation. (PTDC/SAU-GMG/099162/2008, PEst-OE/SAU/UI4013/2011).

P18

The role of the *araL* and *araM* gene products in the toxic effect caused by arabinose in a *Bacillus subtilis* strain defective in AraR

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The accumulation of phosphorylated sugar intermediates has been observed to be toxic for the majority of prokaryotic and eukaryotic cells, however the mechanisms that underlie toxicity are yet to be fully understood. In the Gram positive model organism *Bacillus subtilis*, the ability to utilize L-arabinose is dependent on three intracellular enzymes encoded by the *araA*, *araB* and *araD* genes, which sequentially convert L-arabinose to L-ribulose, L-ribulose 5-phosphate and D-xylulose 5-phosphate respectively, and the latter is further catabolized through the pentose phosphate pathway. These genes are comprised in a large transcriptional unit, the *araABDLMNPQ-abfA* operon, driven by a very strong promoter. Transcription is induced by arabinose and negatively controlled by AraR, a repressor allosterically regulated by arabinose. In an *araR*-null mutant, addition of arabinose to an exponentially growing culture results in immediate cessation of growth. The current hypothesis is that the bacteriostatic effect observed could be due to an increased intracellular level of arabinose, which consequently causes an increase in the concentration of the metabolic sugar phosphates intermediates that are toxic to the cell. Interestingly, the toxic effect is suppressed by a large deletion of all genes downstream of araD. Since neither *araNPQ* (ABC-type transporter) nor *abfA* (arabinofuranosidase) were considered to contribute to the toxic effect, due to its function, the potential candidates are the genes *araL* (sugar phosphatase) and *araM* (dehydrogenase). Here, to investigate their role in the phenomenon of toxicity caused by arabinose, in-frame deletions of both genes were constructed in different genetic backgrounds and the effect of the mutations was analysed in the presence and absence of arabinose. Additionally, we generated and studied *B. subtilis* strains harboring ectopic expression of araL and *araM* under the control of a modified spac promoter. The results indicate that *araL* and *araM* do not play a role in the toxic effect of arabinose observed in an *araR*-null mutant. Thus, we hypothesized that the large deletion of all genes downstream of *araD* might lead to destabilization of the upstream mRNA, causing a decrease in the concentration of the enzymes involved in the catabolism of arabinose, and consequently, leading to a lower the concentration of the sugar phosphates intermediates and suppressing the toxic effect. This hypothesis is currently under investigation.

P19

Contribution of multiple AraR DNA binding sites for the regulation of gene expression and carbohydrate utilization in *Bacillus subtilis*

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Protein-DNA interactions are the primary mechanism of controlling the gene expression at the transcriptional level, affecting the synthesis of messenger RNA molecules of target genes. This step is finely regulated by a number of different factors, among which transcription factors play a key role binding DNA near the transcription start site of genes, in the promoter region, but often also within the region to be transcribed or in distal elements. The *ara* regulon under the control of the transcription factor AraR comprises a set of genes directly, or indirectly involved in the utilization of arabinose, xylose and galactose in the Gram positive model organism *B. subtilis*. This regulon comprises at least thirteen genes located in three different regions of the chromosome. The AraR repressor, in the absence of the effector molecule, arabinose, binds to palindromic sequences (At(T/A)tGTaCGTAcaa(A/T)T) found in the promoter region of the ara genes [1,2]. In this work, we focused on the to study of the cluster located at 256º, which encompasses two transcriptional units, the *araABDLMNPQ-abfA* operon and the abnA gene positioned immediately upstream from the operon. This region, of about 11.7 kb, includes two AraR-binding sites in the *araABDLMNPQ-abfA* operon promoter region [3], one operator in the promoter of the abnA gene [4], and we detected an additional palindromic sequence within the *abnA* coding region. To assess the dynamics of these four operators and to determine their individual role in the regulation of transcription of this *ara* cluster we used in vivo characterization of the wild-type and mutant operators by transcriptional fusions together with in vivo mRNA analysis by RT-PCR. The relative levels of gene expression of *abnA* and of *araA* and *abfA*, the first and last cistrons of the *ara* operon, were quantified and the results will be discussed.
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P20

Cyclooxygenase-2 and Matrix Metalloproteinase-9 Expression in canine melanoma

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The incidence of melanocytic tumours is increasing among canine population. Canine malignant melanoma could have an aggressive behavior, metastasize early in the course of the disease and is resistant to most current therapeutic regimens. Cyclooxigenase-2 (COX-2) is an enzyme that promotes the transition of arachidonic acid in to different prostanoids. COX-2 contributes to invasiveness of cancer through activation of several matrix metalloproteinases (MMPs). MMP-9 is a zinc-dependent endopeptidase that degrades the extracellular matrix, and has been linked to invasion and metastasis. The aim of the present work is to assess the association of the COX-2 expression and the MMP-9 expression in dogs with malignant melanoma. Thirty four canine melanomas tumours, obtained from the UTAD Histopathology Laboratory archives were included. The expression of COX-2 and MMP-9 was carried out by the streptavidin biotin peroxidase complex method with and without bleaching. The immunostaining was semi-quantitatively analysed. Positive COX-2 expression was defined when more than 10% of the tumour cells showed positive staining. The staining intensity was not scored in this method. The expression of MMP-9 was scored in terms of extension and intensity. COX-2 was expressed by 24 of the 34 melanomas. All tumours were MMP-9 positive. A moderate or weak labeling was present in 27 out of 34 melanomas. For MMP-9 extension, 44% of the tumours showed a focal reaction, and 29,4% a diffuse labeling. There was a significant association between the COX-2 expression and MMP-9 extension in canine melanoma (p=0,030), but not for COX-2 and MMP-9 intensity. COX-2 positive tumours had less MMP-9 positive cells than negative COX-2 melanomas. The results showed that COX-2 and MMP-9 were expressed in a high percentage of primary melanoma and their expressions are inversely related. Further studies with MMP inhibitors should be performed to understand the biological meaning of these results.

P21

LMW-PTP characterization in Breast Epithelium cell lines

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Protein tyrosine phosphorylation is a key mechanism through which cells control vital functions such as cell growth, proliferation, motility and gene expression. Low molecular weight protein tyrosine phosphatases (LMW-PTP EC 3.1.3.2) are a group of 18kDa enzymes, with no particular tissue-specificity, that belong to the class II Cystein-Based PTPs, and are represented in the human genome by a single gene, ACP1, located on 2p25.3. ACP1 is polymorphic and has three different alleles (A, B, C). Over-expression of LMW-PTP has been observed in many oncogene-transformed epithelial cells and LMW-PTP overexpression is sufficient to transform non-transformed epithelial cells. However, analysis of a panel of human breast, colon and lung cancer surgical samples did not show increased levels of LMW-PTP mRNA in lung cancer tissue. Aim: To investigate the role of LMW-PTP and its isoforms in different human cell lines, and characterize their differential expression in the context of human cancer. Methods: We have characterized 7 different breast epithelial cell lines (MCF10A, ZR75, MDA-MB-231, MDA-MB-231BO, MCF7, MDA-MB-231BO2, MDA-MB-435), with respect to LMW-PTP genotype, expression and activity. Genotypes were determined by PCR-RFLP. mRNA expression was assessed by Real Time PCR using TaqMan assays. Enzymatic activity was determined spectrophotometrically. Results: In the seven breast epithelial cell lines only three of the six possible ACP1 genotypes were present – AA, AB, BB. Accordingly, the expected decreasing order of fast/slow ratio is BB > AB > AA, corresponding to MCF10A = ZR75 > MDA-MB-231 = MDA-MB-231BO = MCF7 > MDA-MB-231BO2 = MDA-MB-435. However, the calculated mRNA fast/slow ratios (in brackets) were MCF10A (1.000) > MDA-MB-231BO2 (0.511) > MDA-MB-231 (0.368) = MDA-MB-231BO (0.327) > ZR75 (0.228) > MCF7 (0.181) = MDA-MB-435 (0.070) ($p<0.05$). Regarding enzymatic activity, only MCF7 showed a significantly different ($p<0.05$) LMW-PTP activity when compared to MCF10A, with no differences on the other cell lines. Conclusion: The expression of LMW-PTP isoforms is different among breast epithelial cell lines, with an overexpression of the slow isoform in almost all studied tumor cell lines, suggesting that this isoform may be involved in the tumorigenic process. The fact that there was no clear genotype-fenotype relation may indicate epigenetic effects.

P22

Functional analysis of the developmental regulator DmFoxP

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Understanding the genetic and molecular mechanisms underlying phenotypic diversity is a fundamental question in Biology. Changes at different levels of gene regulation, including alternative splicing (AS), have been proposed/shown to contribute to phenotypic evolution. Few examples have established a comprehensive analysis of the different regulatory levels in the functional modulation of a particular gene. We propose to establish such an analysis through the study of the dmFoxP transcription factor (TF) in *Drosophila*. Recent work has shown that this gene undergoes AS generating two different isoforms expressed in the embryonic CNS and in hemocytes. Combining the vast array of genetic and molecular tools available in Drosophila with the preciseness given by in vitro assays, we aim at functionally characterizing FoxP. By analyzing the precise expression patterns of the two FoxP isoforms, their developmental function and their binding properties, we hope to help understanding how AS of TFs can generate phenotypic diversity.

P23

How to modulate your favourite enzyme: examples of synergies between biochemical and structural analysis of ribonucleases

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Enzymes from the RNase II-family are ubiquitous and play an crucial role in RNA metabolism. They are involved in virulence, growth and viability, mitotic control and chloroplast biogenesis. E. coli RNase II is the prototype of this family, and, by structural and biochemical studies, the mechanism of action of these exoribonucleases has been unravelled. The results showed that the residues in the active site of the protein have different roles in catalysis[1]. We also discovered the residue responsible for setting the final end-product, which was also important for RNA binding[1]. Interestingly, we have constructed a protein with is 110 times more active when compared to the wild type, which may have a great biotechnology potential[2]. RNase II is constituted by four domains and this organization is similar for all the other proteins from this family. We studied the role of these domains in E. coli RNase II and RNase R. The two N-terminal and the C-terminal domains are involved in RNA binding and the central RNB domain is responsible for catalysis[3]. Contrary to what is observed for RNase II, RNase R is able to degrade structured RNA. The RNase R RNB domain alone has activity, and in the absence of the RNA binding domains (both CSD and S1 domains), the protein is able to degrade double-stranded substrates even in the absence of a 3' single-stranded overhang. This finding led us to conclude that the RNA binding domains are responsible for the selection of the RNAs that are tagged to be degraded3. We also demonstrate that the S1 domain, and more specifically the C-terminal Lysine-rich region, was involved in the degradation of dsRNAs, probably by helping to unwind the substrate before it enters into the catalytic cavity[4]. Recent evidences suggest that RNase R can act together with other proteins. Due to the importance of these proteins, these results were very important to understand the mechanism of action of RNase II and RNase R, and can be extrapolated to other members of the family.

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P24

The evolution of the RNase II-family of exoribonucleases selected enzymes with peculiar features: examples from Archaea and Cyanobacteria

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Ribonucleases have a central role in the control of gene expression. They are involved in the maturation of functional RNAs as well as their degradation. They are also involved in quality control mechanisms and are crucial to the recycling of ribonucleotides. Exoribonucleases are molecules which cleave the RNA molecules from the 3' or 5' end. RNase II family was shown to be involved in virulence, growth and viability, mitotic control and chloroplast biogenesis. Some organisms present only one homologue from the RNase II-family of enzymes which was shown to be essential for viability. This indicates that they may have a crucial role in the RNA metabolism. Examples of genomes with an unique RNase II-like protein are *Haloferax* (Archaea) and *Synechocystis* (Cyanobacteria). We are interested in studying the mechanism of action of exoribonucleases from the RNase II family in several organisms, namely in archaea *Haloferax volcanii* and in cyanobacteria *Synechocystis* PCC6803. We characterized the activity of the RNase II/R homologue present in both organisms. The results showed that both displayed hydrolytic activity and released nucleoside monophosphates. Our results show that there are many similarities with the *E. coli* homologues (RNase II and RNase R), but a few distinguishing features which may explain the crucial role of these enzymes in RNA metabolism. Namely, in Synechocystis the RNase II-like protein is extremely sensitive to secondary structures[1], and the *Haloferax* protein has different properties according to the temperature[2]. We believe that most of the results can be extrapolated to other important members of this family.
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Symposium 3
Plant Genetics and Genomics

Coordinators: Henrique Guedes-Pinto (IBB, UTAD) and Jörg Becker (IGC)

OC13

Rye supernumerary (B) chromosomes may confer heat tolerance during meiosis

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Rye supernumerary chromosomes, designated as B chromosomes to distinguish them from the normal A complement, have specific sequences important for their accumulation by non-disjunction in the first mitotic division of the pollen grain. One of these sequences, namely E3900, is present in approximately 100 copies on Bs and is also found in 1-3 copies on A chromosomes in rye as well as other cereals. Interestingly, E3900 related sequences are highly conserved and expressed in a tissue and developmental specific manner on plants with and without Bs and may have a role on chromatid adhesion. To further investigate the biological function of E3900, we analyzed the expression of E3900 related sequences as well as meiotic chromosome integrity and structure in 0B and +B meiocytes exposed to 42 oC for four hours. Quantitative Real Time PCR (qRT-PCR) with total RNA isolated from anthers at distinct meiotic stages with primers specific for a heat shock protein indicated that this gene was up-regulated in all plants exposed to heat in a manner specific to the meiotic stage regardless of presence or absence of Bs. Conversely, the expression of E3900 related sequences between heat stressed meiocytes and control plants were significantly regulated in a stage specific manner. Whereas there is a down regulation of the complete as well as the truncated form of E3900 in meiocytes at pre-meiotic interphase, these sequences are highly up-regulated during meiosis. This is evident as a 40 fold increase in the truncated form of E3900 in heat stressed +B plants compared to a 3 fold increase in meicytes with 0Bs. Interestingly this truncated form of E3900 has an open reading frame with a DNA binding motif. Relevantly, cytogenetic analysis indicated a significantly lower proportion of abnormal meiosis in plants with 2B chromosomes. Whereas 0B heat stressed plants had 97% abnormal meiosis, this percentage was reduced to 42% in meiocytes with B chromosomes. Taken together, our results indicate that rye B chromosomes and more specifically E3900 related sequences may have a role in heat tolerance during meiosis.

OC14

Analysis of heat-stress induced genomic modifications in retrotransposon related sequences in *Vitis vinifera*

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Retrotransposons have been shown to be involved in genetic and epigenetic modifications induced by environmental stress in plants. In this study, sequences related to Grapevine Retrotransposon 1 (Gret1) were utilized to determine heat induced genomic modifications in Touriga Nacional, a traditional Portuguese grapevine variety. For this purpose, growing canes were treated to 42 oC for four hours, equivalent to temperatures that grapevine is exposed to in the field. Leaf genomic DNA was utilized to compare the number and organization of Gret1 related sequences between non-treated plants and treated plants immediately after heat stress (0 Hours) and 24 hours post treatment. Primers specific for distinct segments of Gret1 inside the LTR were designed and Quantitative Real Time PCR (qRT-PCR) performed to determine number of copies of Gret1 related sequences in heat treated and control plants. Results indicate significant differences in the number of copies of distinct Gret1 segments in all plants, regardless of exposure to heat. Interestingly, although there were no significant differences in the number of Gret1 Long Terminal Repeat (LTR) related sequences at 0 hours post stress, these were significantly reduced in leaf genomic DNA 24 hours after heat exposure. This is evident as a decrease from an average Â± standard deviation of 228 Â± 90 to 94 Â± 33 of a specific LTR segment in heat stressed plants 24 hours after stress. To further analyze the nature of these alterations, we have performed Southern Blots and PCR based molecular marker techniques involving retrotransposon sequences, namely Retrotransposon Microsatellite Amplified Polymorphism (REMAP) and Inter Retrotransposon Amplified Polymorphism (IRAP). Since the results of these were inconclusive, we are currently performing REMAP, IRAP and Combined with Bisulfite Restriction Analysis (COBRA). This will permit for a more in-depth analysis of heat induced genetic and epigenetic alterations in *Vitis vinifera*.

OC15

Thermospermine - a small polycation with large impact in vascular development of poplar

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Polyamines are low molecular weight polycationic amines present in all living organisms. A small polyamine called thermospermine recently stepped into plant xylem development biology when it was found that the gene encoding for thermospermine synthase, ACAULIS5 (ACL5) is essential for stem elongation and might be acting as a delayer of cell death in differentiating xylem vessels of Arabidopsis plants [1, 2]. Due to the relevance of xylem differentiation in wood formation in trees, we proposed to investigate the role of thermospermine in the vascular tissues of the model tree poplar. By searching the poplar genome databases and then manipulating the candidate gene transcript levels we have found POPACAULIS5, the gene responsible for thermospermine production in poplar. POPACAULIS5 overexpression leads to severe dwarfism in plants in the early growth stages following transformation. However, transfer of these dwarf transgenic plants to a plant growth regulator-free media allows partial recovery of the wild-type phenotype. POPACAULIS5 overexpression greenhouse-grown 2-month-old trees showed a slower production of mature xylem in the stem and, surprisingly, both the expression levels and thermospermine content in stem tissues dropped below those found in wild-type stems but the same was not observed in the leaves. Our observations suggest that thermospermine has a regulatory role in xylem differentiation/maturation in poplar and a negative feedback loop regulation of its production may be active specifically in the stem tissues.
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OC16

DNA sequence analyses of Phytoene Synthase (Psy) gene in Portuguese durum wheat varieties

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Durum wheat (*Triticum turgidum* subsp. *durum*) is a highly economically important crop worldwide for pasta and biscuit production. This species has been target of natural and artificial selection, and has been adapted to a wide range of environments. Recently, a Portuguese research team started to develop a study of Portuguese durum wheat varieties with high production rates in order to identify specific markers for agronomic and economic important traits at the seed level, namely, the production and accumulation of carotenoids which contribute for the yellow colour of the seeds. This trait has been considered a quality indicator for the consumers, being attractive at the moment they bought it, but also because this trait indicates the presence of antioxidants which consume is healthy. The yellow colour derives from the expression of the Psy gene which codes for the first phytoene synthase enzyme of the carotenoids biosynthetic chain.
The goal of the present study is to characterize the sequence of the Psy gene in five Portuguese durum wheat varieties: 'Preto Amarelo', 'Celta', '106', '120', and 'IDYT-MD 10'. For that purpose, we extracted genomic DNA from young leaves using the DNeasy Plant mini kit (Qiagen), and amplified it with several combinations of primers (previously tested by other authors or de novo designed based on the alignment of Psy sequences available on GenBank). After several optimizations of PCR conditions, only 5 combinations of primers amplified fragments of the Psy gene ranging from 800bp to 1500bp. The PCR products were purified and the selected fragments were isolated from agarose gels and purified with suitable kits for further sequencing and analysis. In particular, the combination of the 2500F and 3200R primer pair produced 800bp fragments in individuals from the five durum wheat varieties. DNA sequence analysis revealed the presence of an exon with 150 bp and 2 single nucleotide polymorphisms (SNPs). One of the 2 SNPs was included within the exon and is responsible for the conversion from glutamate (GAG) to the lysine (AAG) amino acid. This alteration is not variety-specific and it could suggest the existence of more than one allele within the locus that codes for the Psy1 enzyme in durum wheat. The remaining fragments are under sequencing.

OC17

Fungal community of chestnut grove: influence of *Hypholoma fasciculare*

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The chestnut tree (*Castanea sativa*) has a great economic importance at national level, mainly due to the fruit value and high quality wood. The presence of the fungus *Hypholoma fasciculare* has been observed in chestnut orchard soils of Trás-os-Montes (Portugal). Although it has been described as being a saprophyte, preliminary studies have shown that *H. fasciculare* could cause serious damages to chestnut trees. On the other hand, it has an expressive antagonist action against other soil-borne fungi present in chestnut groves. Study of *H. fasciculare* influence on chestnut fungal community was performed based on soil samples from groves with different concentrations of *H. fasciculare* sporocarps. The metagenome study was performed by next generation sequencing (454 approach). Results will be presented taking into account the diversity of fungal trophic groups that could be important for chestnut sustainability, namely parasitic, mycorrhizal and saprophytic fungi. These results will contribute to the identification of soil microbial species most affected by the presence of *H. fasciculare*, making it possible to assess the action of this fungus on specific trophic groups, including the benefic mycorrhizal fungi.

OC18

Microsatellite analysis of putative grapevine berry colour mutants

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Microsatellites or Simple Sequence Repeats (SSRs) have been considered the most suitable molecular markers for identification of crop species, particularly grapevine cultivars. With the project GENRES 081 six SSR loci (VVS2, VVMD5, VVMD7, VVMD27, ssrVrZAG62 and ssrVrZAG79) were established for identification of grapevine varieties and belong to the OIV 'Descriptor List for Grape Varieties and *Vitis* Species' (OIV, 2009). The occurrence of different alleles amplified at these loci is indicative of the presence of different cultivars. This work was carried out on groups of accessions of grapevine with same name but with differences in the berry colour, white (W), gris (R) and red (B), collected in the 'Vinhos Verdes' and 'Douro' DOC regions. Confirmation of colour mutants was made through the amplification of the six OIV SSR loci. Based on the alleles detected in the studied accessions, several cases of accessions with the same name, such as 'Carrega Branco' (W) and 'Carrega Tinto' (B), 'Mourisco Branco' (W) and 'Mourisco' (B) and 'Moscatel Galego Branco' (W) and 'Moscatel Galego Tinto' (B) were confirmed as being in fact different varieties. Organisation Internationale de la Vigne et du Vin (OIV), 2009. 2nde édition de la Liste des Descripteurs OIV pour les Variétés et Espèces de Vitis. Ed. OIV, Paris, France.
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P25

Male gametes: Identification of sperm-derived transcripts with a potential role in early embryogenesis and of conserved sperm-cell specific modules

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Arabidopsis thaliana sperm cells (SCs) are known to have a distinct transcriptional profile from pollen and sporothytic tissues and it is probable that transcripts stored in these cells play an essential role during early embryonic development. In fact, SSP (Short Suspensor) transcripts were found to accumulate in the SCs of mature pollen without being translated and delivered to the zygote where SSP protein is produced and acts in the regulation of the first asymmetric division of the Arabidopsis zygote. How the transcripts accumulate in the SCs without being translated is still unknown. Possibly AGO5 and AGO9 proteins, both highly expressed in SCs and known to be involved in gene silencing mediated by small RNAs, can have a role in the translational inhibition of those transcripts by sequestering them at the SCs level and releasing them after fertilization so translation can be initiated. The identification of other sperm-derived transcripts with an essential role in early embryogenesis and the mechanisms towards their translational regulation in SCs are two aspects that we would like to focus on. Based on the transcriptomics analysis of Arabidopsis microgametogenesis, isolated SCs, growing pollen tubes, egg and central cells, synergids and first stages of embryo development 540 genes were identified as having a similar expression profile as SSP and 21 are being tested by reverse genetics in Arabidopsis for their potential role in the early stages of embryogenesis. By now one gene has been identified as a potential candidate. This gene and potentially additional ones will be object of a deeper characterization. To clarify the potential involvement of the sperm-expressed Argonautes in the translational repression of the sperm-derived transcripts, we are performing pull-down experiments of GFP-tagged AGO5 and AGO9 from transgenic Arabidopsis pollen to identify mRNAs and small RNAs that can interact with these Argonautes. Although germ cells can show distinct life styles among species, the molecular pathways towards totipotency are likely to remain conserved. By comparison of the transcriptional profiles of male gametes from Arabidopsis thaliana, Drosophila melanogaster and Homo sapiens we want to identify conserved germ cell specific modules as potential precursors of totipotency. So far we have identified the ortholog genes commonly expressed in these species and are proceeding to the characterization of candidate genes in Arabidopsis and Drosophila.

P26

Establishing the basis for disease resistance genetic studies on Portuguese common bean (*Phaseolus vulgaris L.*) germplasm

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Traditional landraces are unique genetic resources, highly valuable for yield, disease resistance and quality modern crop improvement. Common bean (*Phaseolus vulgaris L.*) was introduced in Portugal more than 5 centuries ago and due to its high organoleptic and nutritional value, it became a popular crop all over the country. Very diverse landraces are still widely grown at the farmers' fields where they are commonly found in intercropping associations with other crops, such as maize (*Zea mays L.*). Nevertheless, several constraints to bean yield stability exist, such as susceptibility to several major fungal diseases. Rusts and powdery mildew fungi, foliar biotrophic pathogens, are among the limiting factors of common bean production. Identification and characterization of sources and mechanisms of resistance to use in plant breeding is regarded as a cost efficient method. Understanding the detected resistance genetic basis will facilitate the resistance handling by the breeders. The study of the genetic diversity existing among the different sources of resistance will be fundamental to select the parental lines to use on the development of contrasting populations needed for the genetic resistance studies. During a field expedition to the Central region of Portugal, members of our team collected several bean landraces adapted to intercropping and low-input farming systems. From those, 45 were screened for genetic diversity with 6 microsatellite markers. Parameters of genetic variation were analyzed, and a Neighbor-joining tree generated, suggesting the existence of two genetically distinct clusters. Additionally, a collection of 92 common bean landraces (7 in common with the described molecular screening) was evaluated for resistance to rust and powdery mildew. Rust infection was analyzed 15 days after inoculation (DAI) with results revealing different types of infection and, although several accessions showed intermediate infection patterns, some landraces presented small necrotic flecks, indicative of resistance. Moreover, regarding the severity of damage caused by both pathogens, the evaluation done 15 DAI suggested that some accessions among the collection have potential to be chosen for genetic resistance studies as they presented low levels of disease severity. The high disease resistance and genetic diversity present among these traditional bean landraces foresee great potential for the improvement of this important national germplasm resources.

P27

Searching for the function of poplar HD-Zip III transcription factors in vascular development: the paralogs HB7/HB8

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Abstract: The *Arabidopsis* genome contains five Class III homeodomain-leucine zipper (HD-Zip III) genes: REVOLUTA, PHABULOSA, PHAVOLUTA, AtHB15 and AtHB8. This family of genes regulates apical embryo patterning, embryonic shoot meristem formation, organ polarity, vascular development, and meristem function. It has been described that all HD-Zip III proteins in land plants are targeted by miRNAs 165/166 for negative post-transcriptional regulation. Given that AtHB8 is expressed in procambial cells of *Arabidopsis* it is thought to be involved in vascular development. Hence, we engaged on understanding the involvement of these transcription factors in poplar, a wood producing model species. Through an in silico analysis, two putative orthologous genes of the HD-Zip III protein AtHB8 have been recognized in the *Populus* genome with domain homology amongst them and also to the *Arabidopsis* conserved domains. This is in agreement with whole-genome duplication events described, with about 8000 pairs of duplicated genes remaining in the *Populu*s genome to the present day. We have found AtHB8 protein to have higher similarity to *Populus* proteins PtHB7 and PtHB8, which are also more closely related to each other than to the remaining HD-Zip III proteins. Presently, it is not known if these paralogs have evolved different functions. We have isolated PtHB7 and PtHB8 cDNAs from both *Populus trichocarpa* and *Populus tremula* x *P. tremuloides* and mutated the miRNA binding site without altering the protein sequence but making it unattainable for miRNA regulation. By generating transgenic *Arabidopsis* plants that express this misregulated form of poplar PtHB7 and PtHB8 genes, we have observed the appearance of aberrant phenotypes, lacking elongation of the inflorescence stems and with diverse abnormalities in leaf polarity, petiole and fruit development. Moreover, similar effects were observed from the manipulation of both paralogs, PtHB7 and PtHB8, suggesting maintenance of the paralog genes function, although the overexpression of PtHB7 had more severe effects. Curiously, these effects are not observed in the *Arabidopsis* AtHB8 overexpression plants, suggesting that poplar genes may have evolved new functions. We are further analyzing the generated transgenic lines to study the expression of other genes possibly involved in the molecular networks implicated in vascular development.
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P28

RAPD evaluation of genomic adjustments in Wheat-rye hybrids

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Polyplidization is known to induce genomic adjustments in parental genomes sharing the same nucleus. Such rearrangements mainly affect repetitive fractions, which correspond to a considerable percentage of plant genomes and may be dispersed or clustered in specific chromosome loci. Several repetitive sequences are known to have functional and structural significance and particularly transposable elements, included in the dispersed fraction of repetitive sequences, have been studied to unravel processes underlying hybrid parental genomes changes. The allopolyploid Triticale was produced through the hybridization between wheat (*Triticum ssp.*) and rye (*Secale cereale L.*). Rye-specific RAPD markers were used to detect rye chromatin in a wheat background, namely a rye specific sequence with ~1450bp - pSc20H - obtained using the primer OP20H 10-mer. This sequence was proved to be dispersed throughout rye chromosomes, except in nucleolar organizing regions and telomeres. The OPH20 10-mer primer was used to study genomic adjustments due to polyploidization through the analysis of PCR banding profiles of rye, hexaploid wheat, octaploid triticale and wheat-rye addition lines. We observed that in triticale one wheat-origin band is missing although all wheat bands are present in the seven wheat-rye addition lines analysed. Contrastingly, both rye-origin bands are observed in triticale, but one of them is missing in all wheat-rye addition lines and the other one (pSc20H) is only present in three of those lines. All triticale parental bands were gel isolated, purified and sequenced revealing high homology with both coding and non-coding sequences of *Secale sp*, *Triticum sp* and *Aegilops sp*, being the former mainly retrotransposon-like sequences. With this work we clearly demonstrate the reliability and consistence of RAPD methodology for the evaluation of parental genomic rearrangements in hybrids, simultaneously allowing the detection of non-coding and gene-rich sequences.

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Evolutive analysis of *Avena* genus: genomic diversity of rDNA sequences

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Avena genus (2n=2x=14) comprises diploid (A and C genomes), tetraploid (AB and AC genomes) and hexaploid (ACD) species. Both A and C genomes have distinct variants and several works suggested that genomes B and D are derived from A genome. Tetraploid species result from hybridization between distinct diploid species, whereas hexaploid ones probably derived from crosses between tetraploid and diploid species. The co-habitation of different genomes in the same nucleus usually induces several epigenetic and genetic alterations mediated by restructuring events. Genomic rearrangements depend on parental genomes ploidy level and sequence type. 45S Ribosomal DNA is composed by coding sequences conserved among species and non-coding polymorphic Intergenic (IGS) sequences and Internal transcribed spacers (ITS) which are important markers for phylogenetic analysis and detection of genomic alterations. Our main goal in this work is oriented to the disclosure of potential genetic diversity of 45S rDNA internal transcribed spacers (ITS1 and ITS2) from one diploid (*A. strigosa*) and two hexaploid species (*A. sterilis* and *A. sativa*), and also for the evaluation of such markers in different accessions of hexaploid species. The control DNA used was pTa71, a plasmid with the entire 45S rDNA subunit from *Triticum aestivum*. ITS1 and ITS2 sequences were amplified through PCR methodology and the resulting bands were isolated, cloned and sequenced. Both ITS1 and ITS2 sequences are very similar between accessions of the each species and between the *Avena* species analyzed, presenting 97 and 100% of homology. Nevertheless ITS1 seems to be more conserved that ITS2 among species. Moreover, it was possible to observe that *A. strigosa* have higher homology with *A. sterilis* than with *A. sativa*. Differences were also observed between all the accessions of *A. sativa* and *A. sterilis* and one of the accessions of *A. sativa* seems more close to *A. sterilis* than to others accessions of *A. sativa*. The analysis of those sequences also contributed to the phylogenetic characterization of the *Avena* species and their accessions.

P30

Identification of proteaginous pea cultivars (*Pisum sativum L.*) using microsatellites molecular marker

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Conventionally morphological descriptors are routinely used for establishing the identity of varieties. This kind of descriptors has some disadvantages, namely most of them are quantitative, controlled by several pairs of genes, and their expression is influenced by environmental factors. Molecular markers have a potential to facilitate this procedure, increase the reliability of decisions, and substantially save the time and space needed for experiments. In this study we intended to identify 20 cultivars of proteaginous pea (*Pisum sativum L.*), registered in the Community Catalog of Varieties, by microsatellites molecular markers. After DNA extraction, seven different loci were analyzed. PCR amplifications were conducted and the resulting fragments were separated on an 3,5% MS-8 agarose gel in TBE buffer, at 90V/h. The gels were analyzed for the presence/ absence of bands and a table with binary code was made. The data were processed with the statistical software NTSYS-pc, using the SIMQUAL module and Jaccard similarity coefficient, followed by UPGMA cluster analysis. With the analysis of six polymorphic loci was possible to distinguish almost all of cultivars. The most informative loci were AD61 and AB53. The cluster analysis of SSR markers separated the pea genotypes into two distinct clusters. The first cluster included the five cultivars: Isard, Cartouche, Audit, Corrent and James. The second cluster included the remaining fifteen cultivars and was further divided in two subclusters. The first subcluster had the Portuguese genotype Grisel and second subcluster contained the remaining fourteen cultivars. In this subcluster Ideal and Alezan had 100 percent similarity. There was a low number of heterozygous loci which is consistent with the nature of self pollinated species. The results showed a high potential and resolving power of SSR markers in distinctness assessment. SSR markers might also be useful in *Pisum sativum L.* germplasm management and genetic diversity studies.
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P31

Olive tree galega cultivar acessions' identification based on RAPD markers

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Olea europaea ssp. europaea (*Oleaceae*) grows in the Mediterranean region. The fruits are used for olive oil and for fruit canning, besides the species is drought and salt tolerant. The Galega cultivar represents 80% of the Portuguese olive area, and it is used in four out of the five Protected Origin Denomination (POD) regions. Despite the large distribution area in the country, cultivar certification is done through phenotypic characteristics, but environmental influence might blur informativeness, and molecular markers could help in clarifying cultivar accessions certification. The 79 putative Galega accessions studied are from a clonal bank, three of them previously identified as Galega, but with different denominations, GGN (Galega Grada Normal), GGS (Galega Grada de Serpa), GGE (Galega Grada de évora), and one as NG (non-Galega). The remaining accessions were propagated from trees considered as belonging to the Galega cultivar, but situated in different parts of the country. We have screened all the accessions with 20 RAPD primers, that amplified 144 bands and 29.2% were polymorphic. The expected heterozygosity was low, as expected in clonal propagated plants (0.10Â±0.01). A Principal Component Analysis (PCA) using the Dice coefficient matrix had 81% and 7% of the variance explained by the first and the second component, respectively. The PCA identified two groups. Group A included all the accessions including GGN, but not #502. Group B included NG, GGE, GGS and #502. The AMOVA (analysis of molecular variance) confirmed a very high differentiation between groups (FST=0.88) and a relative higher homogeneity within groups (12%). Assuming that the GGN individual represents a Galega cultivar pattern, we might assume that all the A group trees are Galega cultivar accessions. Nevertheless, some variability exists within groups, may be due to the accumulation of somatic mutations and/or a putative origin from different but closely related genotypes. Finally, GGS and GGE, together with #502 plot together with the individual identified as not belonging to the Galega cultivar (NG). Group B trees had certainly a very different genetic origin compared to group A genotypes, already reflected in the difference of the names. A future study using a broader sampling and higher discriminant molecular markers, such as microsatellites, would help to unambiguously fingerprinting the Galega cultivar genotypes.

P32

Evaluation of *T. durum* genomic diversity: characterization of repetitive sequences variability in commercial cultivars

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Cereals constitute over 50% of crop production worldwide, belonging to *Triticeae* the most important crops in European agriculture. Durum wheat is one of the cereals with a higher growing demand in Portugal. Wheat commercial cultivars tend to have a low degree of genomic variability, which can constitute a problem in adverse conditions. The rapid climate change observed nowadays is expected to induce a decrease in crops production, thus it is imperative to assess the real level of commercial certified cultivars biodiversity. Inter Retrotransposons Amplified Polymorphism (IRAP) and Inter Simple Sequence Repeat (ISSR) are PCR-based molecular techniques used to survey retrotransposon and/or microsatellite flanking sequence. Generating high resolution profiles those methodologies constitute very powerful tools to characterize species, including access variability between cultivars. To characterize intraspecific *T. durum* variability commercial cultivars Celta, Hélvio and Marialva were studied through IRAP (using primers to Angela retrotransposon LTR) and ISSR (using anchored primers to microsatellite (AAG)6C). For each cultivar different individuals were analyzed to assess commercial cultivars homogeneity. The three cultivars analyzed present distinct IRAP and ISSR banding profiles, including bands characteristic of each cultivar besides the presence of bands common to all genotypes. A higher level of similarity was found between Hélvio and Celta banding profiles which present 90% of common bands. Contrastingly such cultivars only share 83% of bands with cv Marialva. Banding profiles polymorphism within individuals of cvs Celta and Marialva was unexpectedly detected, taking into consideration the reproductive system of this species and the extensive trials associated with cultivars certification. The overall genomic evaluation of distinct T. durum cultivars using IRAP and ISSR lead therefore to the development of important molecular tools, essential for a reliable screening of T. durum cultivars as well as for the assessment of their genomic homogeneity.

P33

Paternally derived miRNAs and their potential impact in early embryogenesis of *Arabidopsis thaliana*

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In *Arabidopsis*, fertilization of the egg cell by one of the two sperm cells is followed by zygote elongation and an assymetric division that will subsequently specify basal and apical cell fate of the developing embryo. Deep sequencing of isolated sperm cells has revealed a rich array of miRNAs, most of which are probably transcribed during pollen development and not inherited from the sporophyte. However, preliminary data suggests they are not fundamental for correct pollen development. What may be then the role for miRNAs in the male germline? In mice, it was recently shown that delivery of miR314c to the oocyte is crucial for the first zygotic division. The delivery of miRNAs by Arabidopsis sperm cells has not been proven, but the transcript of SHORT SUSPENSOR (SSP) was shown to be delivered during fertilization to the egg cell, where it is translated and activates YODA (YDA), a MAPKK Kinase responsible for determining cell fates in the basal embryonic lineage. To address the possibility of miRNA delivery to the egg cell, we are using two different methodologies: A first general approach is to fertilize pollen - expressing artificial miRNAs in the sperm cells targeting GUS - with transgenic lines expressing GUS specifically in the egg cell. Sperm cell artificial miRNA delivery should silence the GUS signal in the egg cell. The second and more specific strategy will be to cross pollen expressing artificial miRNAs targeting genes that are only expressed very early in zygote development and whose mutant phenotypes are known. Silencing of these target transcripts would mean the artificial miRNAs had been delivered during fertilization and are functional.

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Comparative transcriptome analysis of gametes in *Physcomitrella patens*

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Since the beginning of life on Earth, the plant kingdom has transformed the planet, from the creation of habitable environments for other organisms to their influence in global biogeochemical cycles. Directly or indirectly, the world's food production depends on plant products, reason for which the understanding of plant reproduction gains importance. In flowering plants, sexual reproduction comprises a "double fertilization". In this process two sperm cells, delivered by the pollen tube, fuse with the egg cell and the central cell in the female ovule, giving rise to the embryo and the endosperm respectively. During male gametogenesis, the sperm cells responsible for the delivery of the paternal genome to the next generation are generated. A precise and well-coordinated system that regulates dynamic patterns in gene and protein expression is needed in order to assure the genetic integrity of the germline. However, basic questions regarding those regulatory mechanisms are still unclear. Recent methodology to isolate sperm cells from the model plant Arabidopsis thaliana provided a first step by revealing the complex transcriptome of the male gametes. In this study, the moss *Physcomitrella patens* is proposed as model to identify novel genetic components for the differentiation of gametes that could be evolutionary conserved among the plant lineage. As the rest of mosses, *Physcomitrella* belongs to the Bryophytes, a basal lineage of land plants. Its evolutionary position and biological characteristics (e.g. dominant haploid life cycle), the availability of the complete genome sequence and the possibility to perform targeted mutagenesis has made it a very suitable model for genetic evolutionary studies. *Physcomitrella* is characterized by the presence of enclosed reproductive structures: the archegonia (female structure) and the antheridia (male structure) develop at the tip of gametophores covered by leafy structures. After being released from the antheridia, motile sperm cells (antherozoids) swim into the archegonia to fertilize the egg cell. Recently, the high degree of conservation in development genetic regulators among bryophytes and land plants has been shown and albeit clear differences in gamete structure and reproduction mechanisms, we anticipate the existence of conserved components in the regulation of gametogenesis. Through a comparative transcriptome analysis among different tissues of *Physcomitrella*, male gamete specific genes will be identified.

P35

Histochemical and biochemical analyses of aluminum toxicity in rye roots

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Biological membranes are a major target under stress conditions being its integrity and stability possibly considered one of the greatest physiological indicators of tolerance under several abiotic stresses. In acid soils, aluminum (Al) is the main plant growth-limiting factor and its effects tend to be targeted to the plasma membrane of the root tips. Lipid peroxidation and loss of membrane integrity are two types of membrane damage that may be related to Al-enhanced oxidative stress through Reactive Oxygen Species (ROS) formation. The root growth inhibition and the callose synthesis induction are, also, common symptoms of Al toxicity. In this study, Al tolerance of diploid, tetraploid, cultivated and wild species of the *Secale* genus was evaluated. Several staining techniques were performed after 24 and 48 h of Al exposure, to identify cellular changes and callose deposits in the rye root tips. Histochemical staining analyzes allowed the detection of the lipids peroxidation, the localization of Al ions in the roots, the Al accumulation, the callose production and the loss of plasma membrane integrity. The cell membrane damage, associated with Al-induced oxidative stress, was detected analyzing biochemically the lipid peroxidation and electrolyte leakage. The results obtained show a considerable genotypic variability among the *Secal*e species. The histochemical staining analyses showed differences between sensitive and tolerant ryes. It was observed an increase of Al accumulation and a higher callose deposition in the distal part of the transition region of the root apex. The exposure time to Al was determinant in the loss of the membrane integrity as well as in the cell death. However, the date obtained for the lipid peroxidation evaluated after 48 h of Al exposure, show no correlation with electrolyte leakage quantification.
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Symposium 4
Genetics and Biotechnology

Coordinators: Margarida Casal (CBMA, UM) and (Arsénio Fialho IBB, IST/UTL)

OC19

Genetic code engineering generates genetic and phenotypic diversity in the human pathogen *Candida albicans*

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The genetic code of *Candida albicans* is characterized by the reassignment of the leucine CUG codon to serine. Remarkably, the serine tRNA (tRNACAGSer) that decodes the CUG codons is aminoacylated in vivo with both serine (97%) and leucine (3%). In previous studies, we have increased that ambiguity up to 28% and demonstrated that the CUG codon undergoes low levels of reassignment that increase under stress (Gomes et al., 2007). In the light of these results we constructed a series of strains that misincorporate leucine at serine CUG sites on a proteome wide scale at levels that range from 0.64% up to 100%, thus reverting CUG identity from serine back to leucine. A comparative phenomics study of the highly ambiguous and reverted *C. albicans* strains was developed using a screen of 40 growth conditions. Such unique manipulation of the genetic code produced an impressive array of phenotypes, showing that CUG ambiguity can be advantageous under certain physiological conditions, namely in presence of antifungals. Whole genome re-sequencing of these strains revealed accumulation of increasing numbers of single nucleotide polymorphisms (SNPs), in both coding and non-coding DNA regions, and frequent loss of heterozigosity (LOH). SNPs accumulated preferentially in genes involved in cell adhesion and biofilm formation, indicating that *C. albicans* uses its natural CUG ambiguity to increase genetic diversity in pathogenesis and drug resistance related processes. This study provides new evidence for a remarkable flexibility of the *C. albicans* genetic code and highlights unanticipated roles of codon ambiguity on the evolution of genetic and phenotypic diversity.
Gomes AC, Miranda I, Silva RM, Moura GR, Thomas B, Akoulitchev A, and Santos MA (2007) A genetic code alteration generates a proteome of high diversity in the human pathogen *Candida albicans*. Genome Biol, 8, R206.

OC20

Uncovering the Short-Chain Fatty Acids transporters in *Escherichia coli*

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The emergence of pro and prebiotics has revived the importance of short-chain fatty acids (SCFAs) produced by gut bacteria. Although biosynthesis and degradation of SCFAs and other short-chain carboxylic acids (lactate, pyruvate or citrate) are well understood, the transport of these acids is still poorly described. This work aims to establish the mode of action of the SCFAs transporters specifically those belonging to the YaaH family (TC#2.A.96, http://www.tcdb.org/) that has members spread by the 3 domains of life. The *Escherichia coli* YaaH protein is made of 188 amino acids arranged as 6 transmembrane segments (TMS). To our knowledge this protein is uncharacterized and the annotation is based on phylogenetic data. This work explores the role of YaaH as an acetate transporter. *E. coli* holds an already characterized acetate transporter named ActP, member of the Solute:Sodium Symporter (SSS) Family, with 549 amino acids arranged as 14 TMS. We have constructed single and double deletion mutants for yaaH and actP genes in the *E. coli* K12 derivative MG1693 strain. We compared the uptake of labeled acetic acid between the wt and each of these strains. In glucose-grown cells, acetate uptake is only abolished when both genes are knocked out. Single deletions cause a reduction of about 50% in activity (Vmax) compared to the wt, while the double deletion reflects a decrease of the uptake to less than 30%, establishing a role for YaaH as an acetate transporter. We also compared the expression of these genes along time and the results show that they are expressed at different growth stages, with YaaH's activity being predominant in the mid-exponential phase of growth, and ActP during the late exponential and beginning of stationary phase. Cells of the yaaHïDactPD strain complemented with a high copy number plasmid carrying the yaaH gene, displayed a Vmax of 15 nmol acetic acid/mg protein/min and a Km of 0.64 mM acetic acid at pH 6.0. Moreover we assigned a specificity profile to this protein by measuring the labeled acetic acid uptake in the presence of non-labeled competitors. YaaH is a transporter specific primarily to acetate and is inhibited by other short-chain carboxylic acids such as benzoic, formic, propionic and butyric acid.
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OC21

Optimisation of Spectral Codification coupled to SBE for genotyping

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We have previously reported on a strategy that combines Förster Resonance Energy Transfer (FRET) based spectral codification with a single base extension (SBE) reaction for single nucleotide sequence discrimination in solution [1]. As proof-of-concept, we showed that this strategy is capable of unequivocally scoring the allele variants present in solution. To extend the use of this tool to any locus of interest, it would be required the development of an universal approach capable of combining a sequence specific SBE primer to an universal sequence labelled and optimised for spectral codification. Here, we demonstrate the optimisation strategy based on a labelled universal oligonucleotide (donor), the sequence specific primer that allows for incorporation of the complementary modified ddNTP (acceptor). Upon primer extension, the universal donor oligonucleotide hybridises to the specific SBE primer, bringing donor and acceptor into close proximity allowing the FRET to occur between the two. Spectral analysis results in identification of the incorporated acceptor ddNTP, thus of the SNP being assessed. We demonstrate this new genotyping strategy on synthetic targets encompassing a fragment of the c-MYC proto-oncogene.

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OC22

A protein required for efficient host colonization by *Clostridium difficile* is an abundant component of the spore surface layers

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Clostridium difficile is a strict anaerobic spore-forming bacterium, a major causative agent of nosocomial enteric diseases associated to antibiotic therapy in adults, and a growing concern in the community. Spores produced by *C. difficile* are the primary cause of transmission in health care institutions. Despite the central importance of spores in the pathogenesis of *C. difficile*, our knowledge of the spore-related mechanisms involved in host colonization and infection is still incomplete. Here, we show that a 17 kDa cysteine-rich protein, termed Sp17, is an abundant component of the *C. difficile* spore surface. Sp17 is exposed at the spore surface, as suggested by both trypsin susceptibility and immunofluorescence. We show that Sp17 undergoes extensive multimerization both in vitro and at the spore surface. Although synthesis of Sp17 is not restricted to sporulating cells, immunofluorescence studies indicate that during sporulation, the protein is produced under the control of the forespore-specifc regulators ÏƒF, and ÏƒG. Sp17 has been previously shown to be an important for infection in a mouse model. Therefore, our study unveils a link between the spore surface and infection. Moreover, Sp17 is unique to *C. difficile* and can be immunologically detected in spores of a panel of strains which includes several hypervirulent isolates, but not in spores of other *Clostridium* or *Bacillus* species. Hence, in addition with its apparent role in pathogenicity, we suggest that Sp17 can be used as a marker for the rapid immunodetection of *C. difficile* spores.

OC23

Vancomycin-Resistant *Enterococci* from Portuguese hemodialysis patients: molecular characterization of antimicrobial resistance, virulence and clonality

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Hemodialysis patients have played a prominent role in the epidemic of vancomycin resistance. Given the rapid growth of the hemodialysis population, the frequency of infection and the impact of antimicrobial resistance on morbidity and mortality rates, it is of great importance to control the spread of antimicrobial-resistant microorganisms among these patients. Therefore, this study aimed to determine the prevalence of Vancomycin-Resistant Enterococci (VRE) in fecal samples of hemodialysis patients from Portugal and to characterize the isolates regarding resistance to other clinically important antimicrobials, virulence factors and clonality. VRE were detected in four (3.3%) of the 121 analyzed patients. Three of the isolates were identified as *E. faecium*, *E. faecalis* and *E. raffinosus*, exhibiting the acquired vanA type of resistance, and one was identified as *E. gallinarum*. The vanA isolates revealed a multi-resistance phenotype; all isolates showed reduced susceptibility to tetracycline, ciprofloxacin, erythromycin, gentamycin and kanamycin and resistance to ampicillin and quinupristin-dalfopristin was also verified. The antimicrobial resistance genes detected were tet(M), tet(L), erm(B), erm(A) and aac(6')-aph(2''). The *E. faecalis* isolate was the one that presented more virulence factor genes (gelE, cpd, esp, hyl, cylA, cylM and cylL). Through MLST analysis, the *E. faecium* and *E. faecalis* isolates showed to be part of high-risk clonal complexes, CC17 (ST78) and CC2 (ST6), respectively. This study demonstrates that even though hemodialysis patients in Portugal are colonized with VRE at a low frequency, the easy dissemination of these strains is of concern once they constitute a reservoir of resistance genes that can naturally be spread into other, more virulent and potentially pathogenic bacteria.

OC24

Cetuximab as a delivery tool for small non-coding RNAs to colon cancer cells

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We have shown that miR-143 is down-regulated in colorectal cancer and that miR-143 overexpression displays chemosensitizer, pro-apoptotic and anti-proliferative properties in vitro and in vivo. In this study, we aim to develop efficient delivery strategies for the pre-clinical evaluation of miR-143 mimetics in colorectal cancer therapy, using cetuximab as delivery and targeting agent. Cetuximab will be used in the development of a delivery complex consisting in the conjugation of cetuximab with FITC, with subsequent binding of anti-FITC-protamine single chain Fv (scFv) antibody to FITC-labeled cetuximab. miRNAs will then be loaded onto protamine moyeties, which display high affinity for nucleic acids and have already been shown effective in in vitro and in vivo delivery of siRNAs to cancer cells. Cetuximab was FITC-labeled using FluoReporter® FITC Protein Labeling Kit, providing a labeling ratio of ~11 (FITC:cetuximab). In a dose response curve for cetuximab using HCT116 colon carcinoma cells, our results showed that cetuximab does not affect cell viability at concentrations below 2 μM. Immunofluorescence and FACS showed that FITC-labeled cetuximab efficiently labels HCT116 cells, but not SW620 cells that do not express EGFR. For cetuximab-FITC above 0.5 nM, all HCT116 cells are FITC-positive, whereas all SW620 cells are FITC-negative; up to 50 nM, under 3% of SW620 cells were FITC-positive, demonstrating the specificity of EGFR targeting by cetuximab-FITC. We used 5 nM cetuximab-FITC to evaluate delivery of anti-FITC-protamine scFv, and showed specific delivery to HCT116 cells, as evaluated by western blot. The advantage of the development of this delivery tool arises from the combination of the therapeutic agent cetuximab already in use for colorectal cancer treatment together with RNA interference. The delivery strategy presented here is still under development. Nevertheless, the results obtained thus far illustrate the high potential for efficient and specific delivery of small non-coding RNAs to cancer cells, and may provide additional and novel therapeutic tools to expand current options in colon cancer management. (PTDC/SAU-GMG/099162/2008, PEst-OE/SAU/UI4013/2011).

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Quorum-Sensing inhibitors as antimicrobial agents against bacteria belonging to *Burkholderia cepacia* complex

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The *Burkholderia cepacia* complex (Bcc) is an important group of opportunistic pathogens being intrinsically resistant to antimicrobial compounds and detergents. These infections are extremely hard to eradicate and the conventional therapies are not efficient. Therefore, there is a need to develop new treatment approaches. The use of quorum sensing inhibitors (QSIÂ´s) has emerged as a promising therapy since quorum sensing is known to regulate several features that include virulence determinants. Our goal is to study QSIÂ´s as an anti-virulence drug, capable of interfering with Bcc virulence. By doing so, the bacteria will be more exposed to the host immune system, to antibiotics or to detergents, being easily eliminated from the host organism or from surfaces. Even though Bcc strains are able to degrade several compounds, we found that sub-lethal concentrations of the QSI, 4-nitro-pyridine-1-oxide (4NPO), induced a significant decrease of gene expression in cepI and cepR. The homoserine lactone synthase CepI and the transcriptional regulator CepR constitute a conserved QS system, found in all Bcc species, that was shown to regulate several virulence phenotypes. We tested several quorum sensing-dependent phenotypes, as well as, its potential use as co-adjuvant of antibiotics and detergents and as anti-biofilm drug. The results obtained indicate 4NPO supplementation in sub-lethal concentrations, the bacteria presents a reduction of proteases, siderophores, motility and biofilm formation, and is unable to produce exopolysaccharide. 4NPO strongly reduced *B. cepacia* IST408 growth in cultures supplemented with bleach, tween 20, trimethoprim, kanamycin, amikacin and peperacillin. Furthermore, *Galleria mellonella* assays shown that *B. cepacia* IST408 virulence is significantly attenuated when 4NPO is administrated in conjunction with kanamycin and trimethoprim. According to the results obtained, 4NPO seems to be an effective QSI of Bcc species, interfering with several QS regulated phenotypes, and can be used to efficiently enhance the biocide activity of detergents and antibiotics.

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Genetic detection and multilocus sequence typing of Extended-Spectrum Î²-Lactamase-Producing *Escherichia coli* from sheep and beef cattle

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Food-producing animals are the primary reservoir of zoonotic pathogens, and the detection of ESBL producers among *Escherichia coli* strains has increased in recent years. Here we describe a molecular survey aimed determining the population structure and dynamics of ESBL-producing *E. coli* strains recovered from healthy ruminants slaughtered for human consumption in Portugal. For this purpose, a total of 127 faecal samples (73 sheep and 54 beef cattle) were collected. Samples were seeded on Levine agar plates supplemented with cefotaxime. Susceptibility to 16 antibiotics was tested by disk-diffusion method in all recovered isolates and detection of ESBLs was carried out by the double disc diffusion test according the CLSI criteria. PCRs assays were used to study resistance genes, phylogenetic groups and virulence factors. PCR and sequencing methods characterized blaESBL genes responsible for the ESBL-phenotype. In addition, multilocus sequence typing (MLST) was used to identify the genetic lineages of all ESBL-producing *E. coli* strains. ESBL-positive *E. coli* isolates were detected in 9 of the 127 (7%) fecal samples. The Î²-lactamase genes detected were as follows: CTXM-32 in 4 isolates, TEM1+CTXM-1 in 3 isolates and CTXM-1 in 2 isolate. All CTX-M-containing isolates exhibit a multiresistant phenotype, all isolates were tetracycline-resistant and 66.7% were resistant to streptomycin and/or trimethoprim-sulfamethoxazole (SXT). MLST analysis revealed four different STs under 2 clonal complexes (CC10 and CC155). Three of the CTX-M-32-producers presented a novel allelic profile (Adk 6, Fum 41, Gyr 3, Icd 18, Mdh 11, Pur 8, and Rec 14) representing a new ST that was included in the MLST database and registered as ST2526. Concerning the phylogenetic groups, 5 of the CTX-M-containing isolates were classified in the B1 phylogroup and 4 isolates in the A phylogroup. Eight isolates harbored at least one of the virulence factors studied (7 fimA and 1 aer). The presence of CTX-M-producers *E. coli* in food-producing ruminants, beef cattle and sheep, raises important questions in the potential risk factors to public health and above all most of the isolates belong to the clonal complexes CC10 and CC155 that have already been associated with human clinical isolates.

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Characterization of the TetR transcriptional regulator SMc03169 and its contribution to symbiosis between *Sinorhizobium meliloti* and leguminous plants

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Members of the TetR family of repressors are known to control genes whose products are involved in multidrug resistance, catabolic pathways, biosynthesis of antibiotics, osmotic stress and pathogenicity. Here we characterized a TetR member of *S. meliloti* encoded by gene SMc03169. A deletion mutant exhibited lower tolerance to acidic conditions as confirmed by the lower growth at pH 5.5. In addition, antimicrobial susceptibility tests demonstrated increased susceptibility to detergents and oxidative stress agents. The SMc03169 mutant also showed defects in symbiosis with *Medicago sativa*, since the number of pink nodules decreased by approximately 50%. Competitive nodulation experiments showed that the mutant was defective in competing with the wild-type strain for nodulation. Indeed, 99.5% of bacteria recovered from mature nodules whose plantlets had been inoculated with wild-type and SMc03169 mutant at a 1:9 ratio, corresponded to the wild-type strain. In trans complementation of the mutant restored antimicrobial resistance, acidic pH sensitivity and nodulation competitiveness. To understand the role of this putative repressor in cell physiology we performed expression-profiling studies. Comparison of SMc03169 mutant and wild-type transcriptome under symbiotic conditions showed 284 genes differentially expressed. The mutant showed decreased expression of many genes involved in Nod factor and siderophore biosynthesis, phosphate uptake systems, and genes belonging to the heat-shock regulon. Among the genes with significantly increased expression are genes directing succinoglycan biosynthesis, lipopolysaccharide modification and genes whose products are involved in metabolism. Together, our results demonstrate that SMc03169 is involved in regulatory networks targeting cell envelope, metabolism and symbiosis; however its precise role within the regulatory network remains to be determined.

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DNA adduct identification using gold nanoparticles for surface-assisted laser desorption/ionisation time-of-flight mass spectrometry

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Many chemical compounds become reactive towards biological molecules upon cell internalisation and/or biotransformation. Alkylating agents, for example, bind covalently to nucleophilic centres in DNA, affecting structure and dynamics [1]. The persistence of chemically-modified nucleobases (DNA adducts) may result in mutation and alter gene function, which has been considered to be an initiating step of chemical carcinogenesis [2]. Purine nucleobases, mainly guanine, are usually the most frequently altered, thus constituting suitable biomarkers of exposure [3]. Standard techniques for DNA adducts assessment are either time consuming and work intensive (e.g. comet assay) or rely on radiolabelled isotopes (e.g. ^{32}P-postlabeling assay).
Mass spectrometry (MS) techniques, particularly matrix-assisted laser desorption/ionisation time-of-flight (MALDI-TOF), have been increasingly applied for biomolecular analysis [4]. Similarly, surface-assisted laser desorption/ionisation time-of-flight mass spectrometry (SALDI-TOF-MS), where the ionisation principle is similar to MALDI but the organic matrix is replaced by an inorganic surface for energy absorption and transfer to the analyte, has gained recent attention for biomolecular analysis [5]. Nanostructures of various shapes and compositions, including noble metal nanoparticles have been studied for SALDI-TOF-MS [5]. In particular, gold nanoparticles (AuNP) possess unique spectral properties, favourable signal/noise ratio in the lower mass region (<500 Da) and rapid surface temperature increase upon laser irradiation.
Here, we report on the development of a AuNP-SALDI-TOF-MS approach for identification of guanine adducts resulting from glycidamide alkylation. Reduction of the background noise provided by the AuNPs allowed for nucleobase fingerprinting and identification of the presence of DNA adducts in a simple manner.

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Characterization of *Candida albicans* strains with new induced ambiguity

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The ascomycete *Candida albicans* is a normal resident of the gastrointestinal tract of humans and other warm-blooded animals, it is a successful commensal and a pathogen that occurs in a broad range of body sites. It also has high capacity to survive and proliferate in environments with drastic changes in oxygen, carbon dioxide, pH, osmolarity, nutrients and temperature. Surprisingly, it translates leucine CUG codons as serine. Under standard growth conditions 3% of leucine and 97% of serine are incorporated at CUGs, but these values are flexible as leucine misincorporation fluctuates between 0.6% and 5% in response to environmental stress. Remarkably, misincorporation of leucine can be artificially increased up to 28% without visible effects on fitness. In order to determine whether such genetic code flexibility exists at other codons sites, we have produced *C. albicans* strains that ambiguously translate CTA , CTC, CTT (Leucine), ATC (Isoleucine), GCC (Alanine), GTG (Valine), GGA (Glycine), AAG (Lysine), ACC (Threonine) and TAC (Tyrosine) as serine. These strains show small decrease in fitness suggesting that they can tolerate ambiguity at other codons. Some strains had decreased growth rate but an unanticipated increase in protein synthesis rate. These interesting results led us to characterize these ambiguous strains using phenotypic profiling with an array of 25 phenotypic conditions, including temperature, pH, nutritional cues and antifungal drugs. The majority of the strains showed growth advantages on different carbon sources, osmotic stress and oxidative stress. Considering the different amino acid properties, strains mistranslating Leucine, Alanine, Lysine, Threonine and Tyrosine codons were selected for microarray analysis. Gene expression profiling of strains grown at 30ºC revealed deregulation of 32.7% of *C. albicans* genes, while strains grown at 37ºC deregulated 18.8% of its genes. Although the majority of the deregulated genes were specific of each strain, genes involved in amino acid transport and salvage, antifungal resistance, and protein synthesis were horizontally deregulated. These results reveal that different types of ambiguity generate dissimilar responses that may play an important role in adaptation and evolution.

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Molecular characterization of antibiotic resistance in *enterococci* isolates from wild birds in Portugal

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Enterococci are common inhabitants of the gastrointestinal tract of human and animal and are considered indicators for the evolution of antibiotic resistance in different ecosystems. Although these bacteria generally do not cause disease, they might act as reservoir of antimicrobial resistance genes that could be transmitted to other pathogenic bacteria and thus might represent a worldwide problem with large repercussions in public health. Antimicrobial resistance can spread to humans and animals (terrestrial and aquatic) via direct or indirect contact, consumed food/feed and through the environment. Transferable resistance determinants are of particular concern in this respect. Therefore, there is a need to analyze the epidemiology and mechanisms of emergence and spread of antimicrobial resistance. The aim of this study was to determine the occurrence of different enterococcal species in faecal samples of wild birds in Portugal, as well as to determine the phenotypes and genes of antibiotic resistance of these *enterococci*. Susceptibility testing for 11 antibiotics was performed in a series of 124 *enterococci* recovered from different species of wild birds in Portugal (*Fringila coelebs, Carduelis carduelis, Erithacus rubecula, Carduelis chloris, Serinus canaria, Sylvia atricapilla, Turdus mérula, Regulus regulus, Sturnus vulgaris, Coturnix coturnix, Gallinago paraguaiae* and *Turdus philomelos*). *Enterococci* species detected were the following ones (number of isolates): *E. faecalis* (n=49), *E. faecium* (n=36), *E. durans* (n=27) and *E. hirae* (n=12). One vanA-containing *E. faecium* isolate was detected showing high-level resistance to glycopeptides as well as resistance to tetracycline, erythromycin and ampicillin, harboring tet(M), tet(L) and erm(B) resistance genes. From our 124 enterococcal isolates, 51 showed tetracycline resistance (with the presence of tet(M) or tet(L) resistance genes) and 8 showed streptomycin resistance with erm(B) gene. Furthermore, resistance to quinupristin-dalfopristin, teicoplanin, ciprofloxacin, ampicillin, and chloramphenicol was detected in 61, 52, 25, 5, and 1 isolate, respectively. The results acquired in this study are important for establishing policies of prudent use of antibiotics in both humans and animals, as well as to increase the awareness to the risks of inappropriate use of these agents. This study should identify risk factors and will address as far as possible the environmental impact actions to reduce emergence.

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Yeast screening for Ffz-like proteins: searching for new members of a novel family of hexose transporters

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Zygosaccharomyces rouxii and *Z. bailii* are food spoilage yeasts that can grow under harsh conditions restrictive for most yeast species due to their extreme osmotolerance and resistance to weak-acid preservatives. These yeasts are fructophilic, i.e. they consume fructose faster than glucose, whereas the main fermentative yeast *Saccharomyces cerevisiae* presents a glucophilic behavior. A peliculiar sugar transporter, ZbFfz1, was previously characterized as a specific fructose facilitator in *Z. bailii*. In *Z. rouxii*, two similar transporters, ZrFfz1, a specific fructose facilitator and ZrFfz2, a fructose/glucose facilitator were also characterized. These three proteins are phylogenetically unrelated to the Sugar Porter family that includes all the other known hexose transporters, which generally exhibit higher affinity for glucose than for fructose. Surprisingly, the Ffz proteins are closer to the Drug H+ Antiporter family, suggesting the emergence of a new family of sugar transporters. The detection of these peculiar Ffz transporters in at least two fructophilic yeasts points to a possible correlation between their presence and the fructophilic behavior. Searching for proteins similar to the Ffz transporters in available databases we identified several putative proteins with a high degree of homology to the Ffzs (60-64 % protein identity) from *Aspergillus* species and several fungal plant pathogens. All these proteins, that probably belong to the same novel sugar transporter family, reveal several highly specific conserved sequence motifs. Based on these motifs, primers were designed to screen for the presence of FFZ-like genes in other putative fructophilic yeasts. We focused our attention mainly in the genera *Zygosaccharomyces*, *Candida* and *Hanseniaspora* (whose genomes are not yet available). Initial results indicate the probable presence of FFZ-like genes in many of these species, reinforcing the emergence of a new and important family of sugar transporters. This work was supported by the project -Improvement of fructose fermentation by industrial *Saccharomyces cerevisiae* strains. (PTDC/AGR-ALI/112802/2009) and the SFRH/BPD/41812/2007 (FCT) grant.

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Metabolic engineering of lactic acid production in yeast: the role of the monocarboxylate permeases Jen1 and Ady2

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Carboxylic acids are commonly used in food and pharmaceutical industries, however new uses for these compounds are emerging, such as the production of biodegradable polymers from lactate. In this work we aimed at manipulating the metabolism of *Saccharomyces cerevisiae* to produce lactic acid and search for the potential influence of the acid transport across plasma membrane in this process. *S. cerevisiae* W303-1A is able to use L-lactic acid but its production in our laboratory conditions has never been detected. Two monocarboxylate permeases were identified in this yeast species, Jen1 and Ady2. When L-LDH gene from *Lactobacillus case*i was expressed in *S. cerevisiae* W303-1A and in the isogenic mutants jen1Δ, ady2Δ and jen1Δ ady2Δ, all strains were able to produce lactic acid, but higher titers were achieved in the mutant strains. In strains constitutively expressing both LDH and JEN1 or ADY2 a higher outer lactic acid concentration was found when glucose was present in the medium, but when glucose was exhausted, its consumption was more pronounced. These results demonstrate that monocarboxylates permeases expression influences lactic acid production. Ady2 has been previously characterized as an acetate permease but our results demonstrated its additional role in lactate uptake. Overall we demonstrate that monocarboxylate transporters Jen1 and Ady2 are modulators of lactic acid production and may well be used to manipulate lactic acid export in yeast cells.
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TLR9 activation plays an early-protective role against *Paracoccidioides brasiliensis* infection

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Toll-like receptor (TLR) family is responsible for the recognition of pathogen-associated molecular patterns expressed by a wide range of pathogens, playing therefore an essential role in the innate immune response. The differential activation of these molecules, expressed in macrophages and dendritic cells, results in the production of specific profiles of both pro- and anti-inflammatory cytokines, impacting both innate and acquired immune responses. In this work we evaluated the ability of the yeast form of *Paracoccidioides brasiliensis*, the etiologic agent of Paracoccidioidomycosis, to activate TLR2, TLR4 and TLR9 receptors in mouse bone marrow-derived macrophages. In line with previously published our results show that TLR2 plays an important role in the recognition of this fungus, since in its absence a significative decrease of cytokine expression was observed. On the other hand, in the absence of TLR9, responsible for the recognition of DNA CpG motifs, we observed a significant increase in TNF-Î± expression at mRNA level. To assess the role of TLR9 on the establishment of *P. brasiliensis* infection, C57BL.6 males (WT and TLR9 KO) were intravenously infected with 1x106 cells/mL. Our results show that TLR9 KO infected mice die at higher rate during the first 48 hours post infection, resembling a septic-shock. Cytokine quantification in serum of mice, during the first hours of infection, revealed a significant increase of IL-6, a multifunctional cytokine involved in the regulation of the immune response, hematopoiesis, and inflammation. Data on cytokine expression in liver and spleen will be presented as well as the liver immunopathology associated to the infection. Altogether, our results suggest that TLR9 activation plays a protective role against *P. brasiliensis*, mainly in the first stages of infection.

P45

A LysM domain integrates the subcellular localization signal of coat morphogenetic protein SafA of *Bacillus subtilis*

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Endospore differentiation in *Bacillus subtilis* begins with the asymmetric division of a sporangial cell. The larger mother cell then engulfs the smaller forespore. At a late stage in development, the mother cell directs synthesis of a layer of peptidoglycan known as the cortex, which accumulates between the two forespore membranes. The mother cell also controls synthesis of a protective, multilayered protein coat, which is formed at the cytoplasmic surface of the forespore outer membrane. Assembly of the coat requires the action of several morphogenetic proteins, which guide the assembly of the coat components. SpoIVA for example, localizes to the forespore outer membrane and recruits SafA, which serves as the hub protein for assembly of the inner coat. SpoIVA also recruits as SpoVID, which is required for the complete encasement of the spore by the different layers of the coat. SafA contains a LysM peptidoglycan-binding domain at its N-terminal end. The LysM domain is followed by a stretch of 12 amino acids called region A. Region A is essential for proper localization of SafA around the developing spore, because it mediates the interaction of SafA with SpoVID. The isolated LysM domain does not bind to SpoIVA or to SpoVID. However, we show here that point mutations that reduce binding of the LysM domain to purified peptidoglycan in vitro also affect the localization of SafA in sporulating cells. Moreover, we show that SafA fails to localize around the developing spore in cells mutant for the spoVE gene, which are unable to synthesize the spore cortex. The results thus suggest that both the LysM and region A contribute to the localization of SafA. SpoVID also contains a LysM domain, which is required for proper localization of the protein. Surprisingly, the LysM of SpoVID does not bind to purified peptidoglican, and the localization of the protein is insensitive to mutations in spoVE. While the LysM domain of SafA establishes a link between the assembly of the cortex and coat layers of the spore, the LysM domain of SpoVID appears to function by a different, peptidoglycan-independent mechanism.

P46

Studies on gellan gum modification aiming new biotechnological applications

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Over the past decade, a better understanding of the molecular mechanisms and regulatory processes underlying the synthesis of bacterial exopolysaccharides (EPS) has emerged. This knowledge has provided us with powerful tools to engineer bacteria that are capable of not only efficient EPS production but also the production of modified polymers exhibiting unique material properties for specific high-value application. One such exopolysaccharide is gellan gum produced by the non-pathogenic bacteria *Sphingomonas elodea* ATCC 31461. Gellan has been traditionally used for food and pharmaceutical applications, and recently also in the biomedical field as a 3D-scaffold material for tissue engineering due to its unique rheological properties, stability, biocompatibility and biodegradability. The potential for using gellan or gellan-like polymers for such applications is determined by their structure-properties relationships. With the aim of obtaining different gellan polymers we are studying at the molecular level, the mechanisms involved in gellan gum polymerization/chain length determination. In particular, we are determining the role of S. elodea GelC and GelE proteins, which together are homologous of bacterial tyrosine kinases, in regulation of gellan production. In order to achieve this goal GelC and GelE protein subcellular localization is being determined and the effect of several GelE mutations on its enzyme activity and how it affects polysaccharide chain-length distribution is being evaluated. The aim is to manipulate gellan synthesis to produce a new generation of modified gellan polymers possessing different physical properties susceptible to be used as new materials for biotechnological applications.

P47

Linking cell shape to cell division and chromosome structure and segregation by the cytoskeletal-associated RodZ protein of *Bacillus subtilis*

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RodZ is a highly conserved protein of the bacterial core morphogenic apparatus. In *Bacillus subtilis*, deletion of *rodZ* results not only in abnormal cell shapes but also leads to elongation of the nucleoid and to the production of polar minicells containing DNA. RodZ localizes in bands along the longitudinal axis of the cell in a MreB-dependent manner, suggesting that it is part of the actin cytoskeleton. RodZ consists of a cytoplasmic moiety, that contains a predicted helix-turn-helix domain, RodZn, and an extracytoplasmic moiety, RodZc. A single transmembrane domain separates RodZn from RodZc. We have studied the function of RodZn and RodZc. We discovered that the Y32A and L33A substitutions within the presumptive recognition helix (α3) of the HTH motif in RodZn, as well as the Y49A and F53A substitutions outside of the HTH motif (in α4) cause asymmetric cell division. However, only the substitutions in α 4 cause delocalization of RodZ. We suggest that α3 and α4 are used for a protein-protein or protein-DNA interaction important for cell division, whereas α4 is likely to contact MreB. The minicells formed by all the mutants are anucleate. We conclude that nucleoid elongation is not a pre-requisite for asymmetric division. In RodZc we found two residues, G265 and N275, which are important for cell shape determination. These residues are predicted to be essential for the binding to an unknown ligand outside the cell membrane. Overall, our results suggest that RodZ is essential not only for bacterial morphogenesis, but also for the correct positioning of the nucleoid and division septa.

P48

Analysis of msmX and yurJ gene expression, encoding two closely related ATPases proposed to energize multiple sugar ABC importers in *Bacillus subtilis*

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ATP binding cassette (ABC) transporters are integral membrane proteins found in all domains of life that actively transport ligands across biological membranes. This process is essential for several aspects of the cell physiology, such as the import of nutrients and export of waste products. In general, ABC transporters are composed by three protein partners: a nucleotide-binding domain; a membrane spanning domain; and a solute-binding protein. It is estimated that the *Bacillus subtilis* genome, a model Gram-positive organism, encodes at least 78 ABC transporters [1]. In Gram-positive bacteria gene clusters encoding the substrate-binding proteins (BPDs) and the transmembrane domains (TMDs) of sugar ABC transporters might lack the nucleotide-binding domains (NBDs). It has been suggested that one ATPase (NBD) may be recruited by more than one transporter and, in fact, two *B. subtilis* ATPases, MsmX and YurJ, were proposed to energize eight different incomplete ABC transporters involved in carbohydrate uptake [1]. Recently, we showed that the AraNPQ importer is involved in the uptake of Î±-1,5-arabinooligosaccharides, up to at least four L-arabinosyl units, and that this system is energized by the ATPase MsmX [2]. Furthermore, our data assign MsmX as a multipurpose *B. subtilis* ATPase required to energize distinct ABC-type importers, playing a critical role in the efficient utilization of several different saccharides [2]. In this work, based on the observation that MsmX and YurJ are closely related (>55% a.a. identity), we inactivated yurJ to determine if MsmX assists the AraNPQ transporter as a homo- or hetero-dimer. The results showed that this transport mechanism remains fully functional in the yurJ deletion mutant in the conditions tested. Furthermore, we analyzed *msmX* and *yurJ* gene expression by RT-PCR to determine if the two genes are expressed in dissimilar growth conditions, and thus each ATPase able to energize AraNPQ in the presence of different substrates or in different metabolic stages. The results suggest that MsmX and YurJ presence in the cell is dependent on the carbohydrate present in the growth medium. These findings will be discussed.
This work was partially funded by grant PTDC/AGR-AAM/102345/2008 from Fundação para a Ciência e a Tecnologia (FCT) to ISN and fellowship SFRH/BD/73039/2010 from FCT to MJF. [1] Quentin, Y., et al. 1999. J. Mol. Biol. 287:467-484 [2] Ferreira, M.J., and I. de Sá-Nogueira. 2010. J. Bacteriol. 192:5312-5318.

Symposium 5
Evolution and Population Genetics

Coordinators: Isabel Gordo (IGC) and Jorge Vieira (IBMC, UP)

OC25

Dengue Vector in Madeira: geographical origin and insecticide resistance

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Aedes (*Stegomyia*) *aegypti* (Linnaeus, 1762) is the mosquito vector of dengue and urban yellow fever viruses. This invasive species is currently present in Madeira Island (Portugal). It was first detected in October 2005 from unknown origin. Despite the vector control measures immediately implemented, the local mosquito population has been spreading over the island. In this study, we aim to: 1) measure the susceptibility level of *Ae. aegypti* population to insecticides, 2) characterize possible resistance mechanisms involved, and 3) establish the geographical origin of *Ae. aegypti* present in Madeira Island. According to WHO bioassay results, *Ae. aegypti* from Madeira presents high susceptibility to malathion, with mortality rates of exposed females of 99.0%. However, the population showed a high resistance level to DDT and permethrin, with mortality values of 29.4% and 33.3%, respectively. There was also a marked resistance to deltamethrin, with a mortality rate of 65.2%. The CDC bottle assays with alpha-cypermethrin and cyfluthrin showed a high susceptibility of *Ae. aegypti* to these insecticides, with mortality rates of 100%. Sequencing of the voltage-gated sodium channel gene, target site of DDT/pyrethroid insecticides, revealed the presence of only one knockdown resistance (kdr) mutation, V1016I. This mutation showed a reduced allele frequency (6%), which suggests the presence of other insecticide resistance mechanisms rather than kdr. The low levels of nucleotide variability presented by the mitochondrial (mt) genes (COI and ND4) are consistent with a recent introduction of *Ae. aegypti* in the island. Two haplotypes were found. The haplotype network of the COI and ND4 genes suggest two scenarios for the establishment of *Ae. aegypti* in the region: a) two independent introductions corresponding to each haplotype, or b) the establishment of both haplotypes simultaneously at the same colonization event. Considering the geographic distribution of the mt haplotypes and the kdr-mutation detected in the island, and the origin of the migrant human population of Madeira, Brazil and Venezuela are the most likely source-places of the local *Ae. aegypti* population. This study also supports the idea that the insecticide resistance detected in the local population of Madeira already existed in the individuals that colonized the island.

OC26

Chromosome structure is a selectable trait

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Gross Chromosomal Rearrangements (GCRs) have been increasingly found as a particular type of mutation that can contribute to natural variation. Yet their contribution to fitness has not been directly determined. Here we show that GCR are pervasive in natural isolates of *Schizosaccharomyces pombe*, a signature of natural variation that has been previously missed in this species. We further determine the effects of GCR, which were genetically engineered such that the genomic coding sequence is unchanged, in fitness at both the sexual and asexual cycle of their natural life. We find several cases of antagonistic pleiotropy, where GCR with deleterious effects during meiosis have beneficial effects during mitosis. Our results constitute the first direct determination of the fitness effects of these mutations and importantly suggest a mechanism of selection that can explain their polymorphism in nature.

OC27

Plant associations, extreme diversifying selection and phylogeography of astaxanthin-producing yeasts

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Phaffia rhodozyma is a red yeast with biotechnological relevance because it produces astaxanthin, a carotenoid with antioxidant, anti-inflammatory and neuroprotective activities. Although a sexual stage is known, the natural history of this yeast is poorly documented. One of the puzzling aspects of the biology of *P. rhodozyma* is its ecological association with certain tree species, namely *Betula* and *Cornus* in temperate forests of the Northern Hemisphere and *Nothofagus* in correspondent biomes of the Southern Hemisphere. In this work we analyzed in detail such associations in order to shed light on the global population structure and phylogeography of this yeast and on the associations with different trees. To achieve this, a group of 40 isolates was investigated using MLSA of seven genes. The species was resolved into four populations. Two of them were sympatric but highly divergent, one associated with *Betula* and the other with *Cornus* in the Northern Hemisphere. Another two populations were associated with *Nothofagus* in the Southern Hemisphere and were allopatric, one in South America, the other in Australasia. In addition two extremely divergent populations from Australasia were considered to represent two new *Phaffia* species. In general, recombination within and between populations was not detected although a case of hybridization between populations of Australasia and Japan suggested the occurrence of occasional gene flow. Nevertheless, strong inbreeding could contribute to maintain the divergence between these lineages. However, the ultimate cause of this niche-dependent assortment of *Phaffia* genotypes could be diversifying selection, i.e. divergent selection associated with adaptation to the different habitats constituted by *Betula*, *Cornus* and *Nothofagus*. The relaxed molecular clock estimation of the divergence of the *P. rhodozyma* populations indicated a recent (less than 20 MYA) diverge of various *Phaffia* lineages, and consequently the colonization of host trees well after *Nothofagus* (80 MYA) and *Betula* (65 MYA) radiations. Such a recent migration of *Phaffia* and the basal position of the lineages associated with *Nothofagus* suggest that the genus might have an Australasian origin, being originally associated with Nothofagus and more recently with *Betula* and *Cornus*. Moreover, long distance dispersal, rather than vicariance associated with continental drift, is the most likely explanation of the observed distribution of *Phaffia* yeasts.

OC28

RAGE T-374A polymorphism as a protective genetic factor for diabetic retinopathy and macular edema

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Diabetic retinopathy (DR) is the leading cause of blindness in older patients in Europe and US, especially those with poor glycemic and blood pressure control. It is one of the most significant microvascular complications of diabetes mellitus, being characterized by vascular permeability, increased tissue ischemia and angiogenesis. The development of DR seems to be dependent not only on an adequate metabolic control but also on genetic factors. Among the several pathways of glucose metabolism that are implicated in diabetes' vascular complications, the generation of advanced glycation end products (AGEs), which activate the AGE receptor (RAGE), has been associated to the development of DR. The consequence of sustained interactions of AGE-RAGE may trigger RAGE-dependent cellular activation, induce oxidative stress, and promote an inflammatory-proliferative response, leading to vascular dysfunction. The T-374A polymorphism in the RAGE gene promoter has been suggested to regulate gene transcription, and has shown an association with vascular complications in type 2 diabetes, but different studies have reported contradictory results. Purpose: To study the association between the RAGE T-374A polymorphism and DR and macular edema (ME), in a Portuguese population sample. Methods: Population sample: 86 type 2 diabetes patients with and without DR or ME and 43 healthy controls. Outcome measures: RAGE T-374A polymorphism was assessed by PCR-RFLP; lipid profile, hypertension and Body Mass Index (BMI) were determined by standard techniques. Results: The RAGE T-374A polymorphism A allele was more frequent in patients without DR or ME than in patients with DR or ME (45.3% vs 16.3% for DR, $p=0.001$ and 53.5% vs 8.1% for ME, $p=0.008$, respectively). There were no statistically significant differences in the genotypes' distribution between type 2 diabetes patients and healthy controls ($p > 0.05$). Independently of the genotype, hypertension was present in 85.1% (n=41) of patients and was associated with dyslipidemia (57.4%) ($p=0.013$) and higher BMI ($p < 0.05$). Conclusion: Results suggest that in the presence of type 2 diabetes, the RAGE A allele has a protective effect against DR and ME.

OC29

Evidences from a preliminary molecular study of the probable existence of a native population of Scots Pine (*Pinus sylvestris L.*) in Portugal

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Scots pine (*Pinus sylvestris L.*) is the widest distributed conifer, with native populations all through Eurasia. Portugal constitutes the upper occidental limit of Scots pine distribution and the presence of a probable native population in 'Serra do Gerês' (NW), has long surprised botanists and ecologists. This hypothetical outlier in the species geographical range of distribution is confined to a few riparian areas of 'Serra do Gerês' and no attempts to ensure its native origin were made. In the recent years, peripheral populations, such as this of 'Serra do Gerês' have captured the attention of scientist due to their importance in the understanding of the evolutionary history of forest species, especially in the refugial Mediterranean areas. However, the reforestation carried out with this species since the end of the century, using allochthonous plant material, has hampered the location of remnant native specimens. With this work, we aim to study the genetic diversity and relationships among 95 Scots pine individuals, being 55 from two riparian populations of 'Serra do Gerês' and 40 from native populations of Germany, Spain (2 populations) and Sweden. For this, we selected 7 Inter-Simple Sequence Repeat (ISSR) and 8 Random Amplified Polymorphic DNA (RAPD) primers that have been proved polymorphic. The pool of the ISSR and RAPD data obtained was utilized for the construction of an UPGMA dendrogram using the Simple Matching genetic similarity coefficient and SAHN method. Globally, ISSRs and RAPDs were discriminative in the clustering of samples per population (region), and it proved to be useful for the assessment of genetic diversity within and among populations, with a high level of differentiation (FST = 0.35965). The 'Serra do Gerês' individuals segregated into two populations, corresponding to the two riparian populations. Our preliminary results assume the hypothesis of a native portuguese population, widening the southwest limit of Scots pine distribution. Additional efforts will be made using other different molecular marker systems to confirm the hypothesis of Scots pine being autochthonous in Portugal.
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OC30

Are all mth-like genes involved in lifespan determination?

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Methuselah is a putative G protein-coupled receptor (GPCR) showing a recognizable Mth ectodomain and a seven-transmenbrane domain [1]. In *Drosophila melanogaster*, the methuselah (mth) gene controls synaptic efficacy at glutamatergic neuromuscular junction by acutely regulating the neurotransmitter exocytosis [2]. Moreover, in *D. melanogaster*, mth gene has been implicated in lifespan, showing significant associations between naturally occurring Mth amino acid polymorphism and lifespan differences [3], using mutants that encode for a truncated version of Mth protein, which lived about 35% longer [4]; see also review by [5] and using Mth peptide inhibitors [6]. Therefore, mth gene could be a candidate gene implicated in the determination of lifespan. We show that mth gene is present only in the melanogaster subgroup of species and it has an estimated age of 10 million years. In *D. melanogaster*, mth gene belongs to a family comprising 16 genes. Hence, it is of interest to determine if other genes from the mth-like family can influence lifespan too in a species that is distantly related to *D. melanogaster – Drosophila americana. D. melanogaster* and *D. americana* have been diverging for 40 million years and here we show that mth-like genes with the typical Mth protein features are able to explain a considerable amount of the lifespan differences observed in a F2 association study.
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P49

Genetic variation of the wild yeast *Saccharomyces paradoxus* in Eurasia

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Yeasts of the genus *Saccharomyces* are of major importance for biotechnology and for applied and fundamental research. In the last years the increasing number of isolates collected from truly natural habitats has contributed to place these yeasts at the forefront of population genetic studies. In particular, *Saccharomyces paradoxus*, the closest known relative of *S. cerevisiae*, is becoming an attractive model organism for population genetic and genomic studies of natural populations. However, the vast wealth of knowledge that has been gathered contrasts markedly with our ignorance on where and how these important microbes thrive in nature. We used circa 100 natural isolates of *S. paradoxus* obtained in oak woodlands across a longitudinal transept of the Eurasian continent ranging more than 6000 Km, from Portugal to Siberia. Using a set of five neutral polymorphic microsatellite loci spanning four chromosomes, we analyzed the genetic variation and structure of the Eurasian population within a phylogeographic perspective. Estimates of population differentiation were high and suggested the presence of distinct ancestral genetic clusters. Populations from the Iberian and Balkan Peninsulas were assigned to geographically different clusters with significant signal for allopatric divergence. By contrast, northern populations were found to share varying contributions from the ancestral (southern) clusters and did not reveal detectable molecular departures from other populations. Mosaic individuals were only found north of the peninsular populations from Iberia and Balkans. Moreover, the absence of an -isolation-by-distance- pattern suggested a recent origin for the North European populations. Pairwise comparisons of gene flow estimates also indicated that neighboring populations exchanged alleles more frequently than distant ones. However, the average levels of gene flow were low, which can be viewed as an indication that migration may not be sufficient enough to counteract the effect of genetic drift.
Keywords: *Saccharomyces paradoxus*, population structure, gene flow, Eurasia, phylogeography. This work was financially supported by FCT through projects PTDC/BIA-EVF/118618/2010 and PTDC/AGR-ALI/118590/2010 and Ph.D. grant SFRH/BD/77390/2011 (P.M.A.).

P50

Measuring diversity and understanding phylogeography in cider and white wine yeasts

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Saccharomyces yeasts are employed for the fermentation of a myriad of alcoholic beverages since the dawn of civilization. Moreover their well-established laboratorial tractability makes them a suitable model organism for evolutionary and population genetic studies. In spite of this, their natural history is poorly known and their domestication processes are just beginning to being studied. Similarly to *S. cerevisiae, S. uvarum* is also associated with alcoholic fermentations, especially those carried out at low temperatures, namely cider and some types of wine. In *S. cerevisiae* a strong relationship between genetic divergence and ecology has been observed, with two identified domestication events, for wine and sake[1]. On the other hand, phylogeographic patterns are weak in this species. Since such information is virtually lacking for *S. uvarum*, we explored the phylogeography and ecological patterns of *S. uvarum* by studying 50 strains isolated in Eurasia, North and South America and Australasia from several ecological sources including nature and fermentation processes. The phylogenetic analysis of three nuclear genes allowed the identification of three main clades, two of them showing geographical associations with Patagonia and Australasia and a third one including strains from all regions except Australasia. Although the recognition of a Patagonian population distinct from the population of Northern Hemisphere was not supported by microsatellite typing, the separation of the Australasian population was corroborated. Therefore, and contrary to what was found for *S. cerevisiae*, no evidence for domestication was detected and, instead, a strong phylogeographic signal was observed. Moreover, the Australasian population presented genetic and phenotypic divergence from the remaining populations and mating studies showed partial reproductive isolation. Although these features have not been observed for *S. cerevisiae*, in *S. paradoxus*, which is only found in the wild, partial reproductive isolation between populations from different continents has been documented[2]. Furthermore, the ancestry of the Australasian and Patagonian populations was suggested due to the presence of private alleles in high frequencies in both populations.
This work was financially supported by FCT through projects PTDC/BIA-EVF/118618/2010 and PTDC/AGR-ALI/118590/2010. Keywords: *Saccharomyces uvarum*, yeast, evolution, phylogeography, domestication, population genetics.

P51

Epigenetic variation in species of the *Limonium ovalifolium* complex (*Plumbaginaceae*)

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Cell-cell communication is necessary for the crosstalk between cells that constitute multicellular organisms. Adhesion molecules have been assigned an important role in melanocytic tumor progression. E-cadherin is a calcium-dependent cell-cell adhesion molecule expressed by melanocytes and responsible for their adhesion to keratinocyte. The aim of this study was to investigate the immunoexpression of E-cadherin in canine cutaneous melanocytic tumors and its correlation with tumor diagnosis. The expression of E-cadherin was evaluated by immunohistochemistry in 30 canine cutaneous melanocytic tumors, and scored in terms of labeling location (membranous, cytoplasmic and heterogeneous), extension and intensity. Only 2 tumours (malignant melanomas) were negative for E-cadherin. Membranous E-cadherin staining was generally absent in dermal nests of melanomas, whereas it was present in a high proportion of benign lesions (melanocytomas). E-cadherin immunoreactivity in malignant melanomas displayed a heterogeneous or diffuse cytoplasmic staining. However, the differences were not statistically significant (p=0,055). Both benign and malignant tumours had a diffuse moderate or strong reaction. E-cadherin staining was detected in all benign and almost all malignant lesions, however with a different pattern in labeling localization. These findings indicate that changes in the cellular expression localization of E-cadherin could occur in melanocytic lesions and may reflect different stages in their progression.

P52

Genetic variation of haptoglobin in several ethnic groups may be associated with environmental factors related with salt availability and malaria

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The genotypic variants 1.1, 2.1 and 2.2 of haptoglobin (Hp), an acute phase protein, might influence the inflammatory and vasomotor responses, dependent on environmental availability of salt and agents such as Plasmodium, implicated in malaria. The allelic 1 variant has been associated with increased sensitivity to salt of blood pressure (BP). Regarding malaria, the data are contradictory. The aim of this study was to investigate these associations in some populations. Material and Methods: In a sample of 2397 subjects from Europe (Portugal and France), North (Canada and United States) and Central (Honduras) Americas, Africa (Mozambique) and Asia, we studied the distribution of Hp. The phenotypes were determined by PAGE and salt sensitivity in 311 subjects, by standardized methods. In 261 of these were determined the sub-phenotypes of salt sensitivity that is, low rennin and non-modulators (both salt sensitive) and insensitive to salt. Statistical methods were X square and logistic regression. Results: The frequency of Hp 1 allele ranged from 0.6 in Mozambique (Africans), 0.56 in Honduras, 0.42 in Portugal (Caucasians), 0.35 in the USA (Caucasians) to 0.3 in Asians, being the differences in the distribution significant (USA-Caucasians vs. Mozambique-Africans, $X^2 = 72.4$, $p < 0.001$; Portugal-Caucasians vs. Mozambique-Africans, $X^2 = 60.9$, $p < 0.001$; USA and France-Africans vs. Mozambique-Africans, $X^2 = 5.96$, $p = 0.05$). In Africans of Mozambique, regardless BP, Hp1.1+2.1 subjects were more sensitive to sodium compared to 2.2 ($p = 0.01$). In the study of sub-phenotypes of salt sensitivity, it was found that this was dependent on the phenotypes 1.1+2.1 in African descendent subjects no modulators and low rennin (OR = 4.7, CI :1.3-16, 6) and of phenotype 2.2 in Caucasians no modulators ($X^2 = 3.84$, $p = 0.05$). Conclusions: The phenotypes that modulates the salt sensitivity of BP, seems to be different between ethnic groups, being the allele 1 variant predominant in Africans and African-Americans who were associated with sensitivity to salt, and allele 2 variant in homozygosis in Caucasians, associated with salt insensitive status. Those effects may be dependent on the environmental salt availability and also of natural selection caused by malaria agents, not only on the African continent but also in Central America.

P53

Evolution of the sex-determining genes in sexual and asexual red yeasts species

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Fungi have evolved a rich repertoire of lifestyles, mating strategies and sex-determining systems with no parallel in other eukaryotic lineages. Red yeasts, in particular, are an early-diverged group of pigmented basidiomycete yeasts comprising sexual and asexual species. Sexuality in this group is based on two compatible mating types (or sexes) and sexual identity is determined by MAT loci that encode homeodomain transcription factors (HD1/HD2), peptide pheromones and their receptors (P/PR) [1]. Taking advantage of their similar ecological and physiological properties and the notorious co-existence of sexual and asexual species in the same phylogenetic lineage, we explored possible causes and/or consequences for the asexuality in this group, by investigating the integrity and evolution of the MAT genes. After surveying 18 sexual heterothallic (self-sterile) and homothallic (self-fertile) species and 16 asexual species, we found that PR homologues were present in most of the sexual and asexual species, while sequence comparisons using whole-genome data indicated that synteny along the pheromone receptor region is reasonably conserved within the same mating type in different species. For the HD homologues obtained from two selected lineages, likelihood methods suggest two different patterns of evolution: (i) diversifying selection acting on the self/non-self recognition region of the HD proteins seems to promote diversity in sexual species, while (ii) rapid evolution of these proteins in asexual species is more consistent with relaxed selection. Our results suggest that events of loss of sexuality seem to be recent, given the lack of a footprint of gene loss and decay, but appear to be frequent and not uniformly distributed within the red yeasts. This scenario of loss of sex associated with relaxed selection on MAT genes could eventually promote the emergence of short-lived asexual lineages and new ecological adaptations, but does not seem to generate exclusively asexual phylogenetic lineages that persist for a long time.
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P54

Genotype and allele frequencies of CYP2D6*4 and CYP2C19*17 single nucleotide polymorphisms in a Portuguese Population

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Some cytochrome-P450 isoforms, such as CYP2D6 and CYP2C19, play a central role in the biotransformation of xenobiotics, including several drugs like antidepressants, antipsychotics and anti-tumoral drugs. The CYP450 gene family is highly polymorphic and, according to the implications that these polymorphisms have on drug metabolism profile, individuals are classified as UM (ultra-rapid metabolizers), EM (extensive metabolizers), IM (intermediate metabolizers) and PM (poor metabolizers). In this context, a detailed study is necessary in order to correlate these polymorphisms with the biotransformation of xenobiotics, both in clinical settings and in the forensic context. The present work aimed at predicting the incidence of certain genetic variations in the Portuguese population. We studied two polymorphisms, CYP2D6*4 and CYP2C19*17, which affect cytochrome-P450 enzyme encoding genes. The CYP2D6*4 allele is the most common variant in Caucasians and is the most frequent non-functional allele in the phenotype, leading to decreased functionality of the enzyme. In contrast, CYP2C19*17 has been associated with increased enzyme function. With this purpose, genotyping was performed in samples collected from volunteers of our institution. Other parameters were analyzed, namely the feasibility of using different biological matrices to perform such genotyping. The methods used involved genomic DNA extraction from whole or dry blood, buccal epithelial cells and hair. Different extraction methods were used, namely phenol-chloroform, salting-out and a commercial kit. The best results were obtained with the commercial kit, using whole blood, both at qualitative and quantitative levels. For the remaining matrices, buccal epithelial cells were those that allowed the extraction of a higher amount of DNA and less contamination. However, all of them proved to be feasible for the genotyping, despite the scanty amount of DNA obtained when compared to the whole blood. After genomic DNA extraction, samples were analyzed through PCR-RFLP and HRM, in order to assess the allele and genotype frequencies of the polymorphisms under study. The results allowed the identification of different genotypes for the CYP2D6*4 and CYP2C19*17 polymorphisms. In the future, these results may constitute important tools in the establishment of individualized treatments, adjusted to the individual profil, optimizing the therapeutic response, minimizing adverse reactions and preventing cases of toxicity.

P55

Adaptation of *Escherichia coli* to macrophages

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Microbial populations constantly deal with host innate immune defenses. We use experimental evolution to show that in the presence of the macrophages commensal Escherichia coli populations adapt very rapidly to circumvent immune recognition. Such fast adaptation is due to transposon insertions, which lead to phenotypes characteristic of pathogenic bacteria, namely small colony variants (SCV) and mucoid (MUC) clones. Whereas SCVs showed increased survival intracellularly, MUCs, which overproduce exopolysaccharides, evolved the ability to escape engulfment by macrophages. MUCs also bear a general advantage when facing predators, such as protozoa and phages. Strikingly, MUC cause a pathological effect in vivo, and SCV have a clear fitness advantage in the gut. Finally, we reveal that a transition from SCV to MUC clones occurs both in vitro and in vivo, a finding that links these two phenotypes of pathogenic bacteria. These results demonstrate the remarkable fast pace at which bacteria can evolve to escape the first line of defense by the immune system and have important implications for Human Health.

P56

Contribution to the genetic characterization of *Fasciola hepatica* populations in Portugal

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Understanding the genetic variation of *Fasciola hepatica* populations has important implications for epidemiology and effective control of fascioliasis. The aim of the present study is to provide the first genetic characterization of *F. hepatica* isolates from different regions of Portugal. Forty seven adult F. hepatica were collected from the livers of naturally infected cattle and sheep from ten districts of Portugal. Genomic DNA was extracted and analyzed using RAPD-PCR and RFLP techniques. RAPD-PCR with a set of 12 arbitrary primers, AP1, AP2, AP3, AP5, AP7, AP8, AP9, AP11, AP12, AP13, AP15 and AP16, was used to estimate genetic intraspecies variation. All the primers have shown their limitations and were not able to distinguish *F. hepatica* isolates from different regions of Portugal. OSA9, OSA10 and OSA11, a set of primers usually used to discriminate cattle and sheep isolates, did not show any different pattern within *F. hepatica* isolates of each host. *F. hepatica* ITS region (995 bp) and partial sequences of mithocondrial genes nad I (382 bp) and cox I (429 bp) were successfully amplified for all samples. The PCR products were further differentiated using the restriction endonucleases AluI, HinfI and RsaI. The digestion pattern was the same for all the isolates. *F. hepatica* isolates from different regions of Portugal did not show any noteworthy genetic variations. All the 47 isolates (39 cattle and 8 sheep) came up with an identical RAPD-PCR and RFLP profile. No significant intraspecific variation was seen in the Portuguese *F. hepatica* isolates for mithocondrial genes nad I and cox I and ITS region, indicating a low genetic diversity of this parasite in Portugal.

P57

Evolution under small versus large populations in the light of Fisher's geometrical model

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What determines the rate of fitness change over time is one of the major questions in evolution. A key factor in the evolutionary process is the selective effect of a mutation, and this depends on the environment and genetic background. Furthermore, for a given mutation with a given effect its fate depends on the effective population size. Here, we manipulate both population size and genotype composition and follow the evolution of *Escherichia coli* to uncover a very simple rule: the rate of fitness change is negatively correlated with the fitness of the genotype in which new mutations appear. Such pattern is expected under population genetics models that include epistatic interactions between mutations. We therefore analyze the observed patterns in light of Fisher's geometrical model (FGM), which is based on stabilizing selection operating for a number of phenotypic traits. We find that this model can capture the general patterns observed and infer the parameters that best fit the data, using Approximate Bayesian Computation. We find that a genomic mutation rate of 0.007 mutations per generation per individual, an effect size of mutations of 0.005 and phenotypic complexity 6. Under this model we find the same pattern of epistasis as observed in the experiments. Furthermore, the type of epistasis associated with FGM is neither negative nor positive. Rather it leads to an increase in variance and the pattern of antagonism comes from the asymmetry in the probabilities of fixation of beneficial and deleterious mutations. We show how a change in variance due to the genetic background can imprint a pattern of antagonistic epistasis.

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Evaluation of Genetic Diversity of *Pinus sylvestris L.* Mature Stands in Portugal

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Pinus sylvestris L. (Scots pine) is the most widely dispersed conifer throughout Eurasia, ranging from arid mountainous areas of Spain and Turkey to sub-arctic forests of north Scandinavia and Siberia. Although there are some populations of Scots pine in Portugal, no molecular studies were yet presented and no reports exist about the origin of the seeds used in the afforestation of these stands. The demand of a proper germplasm management and a suitable seed selection for forest plantations justifies the need of studying its genetic variability and adaptation to local conditions, mainly for the sustainable use of forest genetic resources. In this study we intend to evaluate the intra- and inter-populations genetic diversity of Scots pine, using the Inter-microsatellite (ISSR) and Random Amplified Polymorphic DNA (RAPD) markers. We studied a total of 204 individuals from 9 populations, comprising 5 Portuguese populations located in Trás-os-Montes; Peneda; Serra da Estrela; Pedra Bela; and Vinhais; and 4 foreign populations from Germany; Sweden; and Spain (2 populations). Several primers were previously tested by us for both marker systems. However, only seven ISSR and eight RAPD primers produced amplification and/or polymorphism. The percentage of polymorphism achieved by the ISSR and RAPDs markers was 100%. The ISSR and RAPD data were used to estimate the genetic relationships among the nine populations, where it was detected polymorphism among and within regions. This pool of data was utilized for the construction of an UPGMA dendrogram using the Simple Matching genetic similarity coefficient and SAHN method. Both marker systems allowed the clustering of individuals per region, correlating the genetic variability with their geographical location, demonstrating to be suitable for the assessment of genetic diversity within and among stands. Additional efforts will be made using other different molecular marker systems to complement the data already collected aiming an accurate evaluation of the genetic variability included in Portuguese *Pinus sylvestris* mature stands. Acknowledgements: This work was supported by the FCT project PTDC/AGR CFL/110988/2009.

Notes

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