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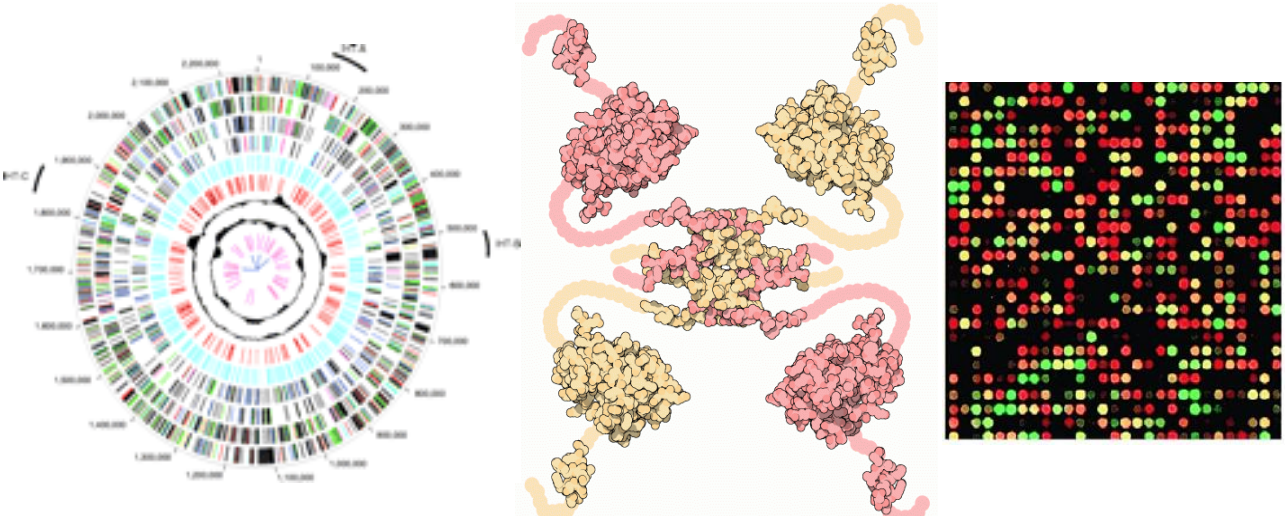

GENÓMICA Y PROTEÓMICA

OCTAVA REUNIÓN DE LA RED VALENCIANA DE GENÓMICA Y PROTEÓMICA

8th Meeting of the Valencian Network for Genomics & Proteomics

Salón de Actos del Centro de Investigación Príncipe Felipe.
11 de Noviembre de 2010

PRINCIPE FELIPE
CENTRO DE INVESTIGACION

Patrocinadores:

MÁSTER UNIVERSITARIO EN BIOLOGÍA MOLECULAR, CELULAR Y GENÉTICA

CONSELLERIA D'EDUCACIÓ

Prometeo/2010/001 i /005

PROGRAMA DE LA JORNADA
Workshop program

9.15 h. **Meeting Welcome**.
José E. Pérez. *Departamento de Bioquímica y Biología Molecular. Universitat de València*.

9:30 h. Invited Scientific Presentations. Session I: Genomes and Transcriptomes: Chairman: Juanjo Calvete.

9:30 h. Simon Wagstaff. Liverpool School of Tropical Medicine
Transcriptomics of the snake venom gland

10:15. R. Gil. Institut Cavanilles de Biodiversitat i Biologia Evolutiva. Universitat de València.
Towards the synthesis of minimal (living!) cells

11:00. Coffee-break and visit to posters.

11:30. Session II: Proteomics and Systems Biology. Chairman: Ismael Mingarro

11:30. Markus Rehm. Dept. of Physiology & Medical Physics. Royal College of Surgeons in Ireland
Biophotonic and systems biological investigation of cell death signaling

12:15. Monique Slijper. Biomolecular Mass Spectrometry and Proteomics Group. Utrecht University
Proteomics strategies to explore membrane protein complexes.

13:00. Time for lunch and visit to posters.

15:00. Session III: Functional Genomics in Yeast. Chairwoman: Susana Rodríguez-Navarro.

15:00. Sebastián Chávez. Departamento de Genética. Universidad de Sevilla.
Control of transcription elongation across the yeast genome.

15:45. Mordechai Choder. Faculty of Medicine. Technion - Israel Institute of Technology.
Gene expression is a circular system.

16:30: Poster session. Beer and snacks available.

18:00: End of the meeting.

Plenarias
Plenary sessions

TRANSCRIPTOMICS OF THE SNAKE VENOM GLAND

S.C. Wagstaff*[1]*, *J.J. Calvete*[2] *and R.A. Harrison*[1]

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Snake venoms are extraordinary complex, typically containing several tens (sometimes hundreds) of some of the most tissue destructive components in nature. Determining the composition of snake venoms is critical to our understanding of, and developing rational treatment for, the pathology associated with snake envenoming. Structure/function characterisation of venom proteins is especially challenging because the inference of the results is complicated by the molecular diversity of venom components and the extraordinary expansion, by gene duplication and divergence, of some of the most pathogenic venom protein families.

Our approach to unravelling the composition of snake venoms involves combining analyses of the venom gland transcriptome (cDNA libraries) and the venom proteome. We use a variety of bioinformatic tools to process venom gland EST sequencing data (typically 1000 ESTs) using highly stringent (isoform-distinguishing) clustering and assembly techniques into a partial transcriptome. *In silico* translation and annotation methodologies (using a range of bioinformatic tools such as gene ontology (GO) annotations, Blast and motif annotation) are then applied to predict the mature venom proteome. This is then experimentally correlated to the true venom proteome using a combination of proteomic and molecular approaches.

The difficulties of dealing with data from this non-model organism and the significance of our findings to our understanding of snake venom biology and the design of rational snake bite immunotherapy will be discussed.

Funded by the Biotechnology and Biological Sciences Research Council (BB/F012675/1) project grants (RAH and SCW)..

TOWARDS THE SYNTHESIS OF MINIMAL (LIVING!) CELLS

Rosario Gil

Institut Cavanilles de Biodiversitat i Biologia Evolutiva. Universitat de València. (Rosario.gil@uv.es)

Synthetic biology is a re-emerging multifaceted research program that, more than any other biological discipline, recreates nature. Its protocell, DNA-based device construction, and genome-driven cell engineering views have gained increased interest due to recent spectacular technical and conceptual advances in the biological sciences. In this talk we will review the state of the art of the last approach, paying particular attention to what we can learn on naturally reduced genomes from either symbiotic or free living bacteria. Different minimal hypothetically viable cells can be defined on the basis of several computational and experimental approaches. Different projects aiming at simplifying living cells are under way, and they are complemented with projects for making synthetic genomes for minimal cells, based on recent advances for *de novo* synthesis of long DNA sequences. On top of it, the effort to create artificial living systems drives new exciting discoveries and theories regarding the description, understanding and manipulation of life which will deliver important technological benefits.

References:

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BIOPHOTONIC AND SYSTEMS BIOLOGICAL INVESTIGATION OF CELL DEATH SIGNALING

Markus Rehm

Department of Physiology & Medical Physics, Royal College of Surgeons in Ireland, RCSI York House, York Street, Dublin 2, Ireland. phone: 00353 (0)1 4028563; email: mrehm@rcsi.ie

Intracellular cell death signalling networks comprise dozens of simultaneous variables, amongst them protein concentrations, trafficking rates, and transmembrane potentials. Decades of biochemical research identified, isolated and precisely characterized many protein components of these networks. To understand their biological function, quantitative single-cell photonics can provide physiologically highly relevant data on intracellular signalling dynamics in time and space. However, only a limited number of cellular parameters can be detected in parallel. To overcome these limitations and to analyse cell death execution on a systems level, we feed both biochemical and imaging data into computational models of cell death execution and can quantitatively predict experimental cellular responses. Here I will (i) demonstrate the main principles of quantitative photonics and systems modelling approaches, (ii) describe how their combination can significantly extend the explanatory power of experimental studies, and (iii) demonstrate how this approach can be employed to inform and guide subsequent research strategies towards identifying suitable treatment paradigms for highly resistant human cancers.

Funded by Science Foundation Ireland, the Health Research Board Ireland, and the National Biophotonics and Imaging Platform, Ireland, funded by the Irish Government's Programme for Research in Third Level Institutions, Cycle 4, National Development Plan 2007-2013.

PROTEOMICS STRATEGIES TO EXPLORE MEMBRANE PROTEIN COMPLEXES

***Monique Slijper*[1,2]**

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[2] Netherlands Proteomics Centre, the Netherlands

The analysis of proteins in biological membranes forms a major challenge in proteomics. We are particularly interested in membrane protein complexes and the changes therein. Therefore we optimized Blue-native PAGE for specific cellular systems. First, we explored this technique for the erythrocyte membrane, for which many membrane protein aberrations are known that result in hemolytic anemia, however, the origin of numerous disorders is not known to date. We assessed the potential of using CyDye labeling to relatively quantify the membrane proteins. Our final goal was to determine if this approach is suited to detect protein level changes in disordered erythrocyte membranes, and we could successfully confirm that erythrocyte spectrin levels were dramatically decreased for a hemolytic anemia patient. Second, we investigated the effect of anaerobiosis on *Saccharomyces cerevisiae*, on the formation of respiratory chain and other protein complexes. For this purpose, we chose an approach in which native mitochondrial membrane protein complexes were separated by 1D BN-PAGE, which was combined with quantitative analysis of each complex subunit using stable isotope labeling. Surprisingly, we discovered that under anaerobic conditions, where the yeast respiratory chain is not active, the respiratory chain super complexes were still present, although at reduced levels. Pearson correlation analysis showed that the composition of the mitochondrial complexes was unchanged under aerobic or anaerobic conditions, with the exception of complex II. In addition, this latter approach allowed screening for possible novel complex interaction partners.

1) van Gestel RA, van Solinge WW, van der Toorn HW, Rijksen G, Heck AJ, van Wijk R, Slijper M. (2010) Quantitative erythrocyte membrane proteome analysis with Blue-Native/SDS PAGE. *J Proteomics* 73(3), 456-65.
2) Helbig AO, de Groot MJ, van Gestel RA, Mohammed S, de Hulster EA, Luttik MA, Daran-Lapujade P, Pronk JT, Heck AJ & **Slijper M**. (2009) A three-way proteomics strategy allows differential analysis of yeast mitochondrial membrane protein complexes under anaerobic and aerobic conditions. *Proteomics* 9, 4787-98.
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CONTROL OF TRANSCRIPTION ELONGATION ACROSS THE YEAST GENOME.

Gómez-Herreros, F.[1], V. Pelechano[2], Rodríguez-Gil A.[1], M.D. de Miguel[1], M. Morillo-Huesca[1], J. García-Martínez[3], M.C. Muñoz-Centeno[1], J.E. Pérez-Ortín[2,3] and ***S. Chávez***[1].

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Transcription elongation by RNA polymerase II in yeast has been considered an invariant non-regulated process. Using a combination of ChIP-on-chip and genomic run-on approaches, we have found that the proportion of transcriptionally active RNA polymerase II (active versus total) present throughout the genome is characteristic for some functional gene classes, like those related to ribosomes and respiration. This proportion also responds to regulatory stimuli, like those transduced by protein kinase A, and in the case of cytosolic ribosomal-protein (RP) genes it is mediated by the silencing domain of Rap1. We have found that this inactive form of RNA polymerase II, which accumulates all along the body of ribosomal protein genes, is phosphorylated in the Ser 5 residue of the CTD but is hypophosphorylated in Ser 2.
We have also determined the relative levels of active RNA polymerase II present at the 3' and 5' ends of 261 yeast genes by run-on. The results obtained indicate that the 3'/5' run-on ratio varies among the genes studied by over 12 $\log_2$ units. The correlation between the 3'/5' RNA polymerase II ratios measured by run-on and those obtained by ChIP is poor, although RP genes present exceptionally low ratios in both cases. We detected a subset of elongation-related factors that are important for maintaining the wild-type profiles of active transcription, including DSIF, Mediator, factors related to the methylation of histone H3-lysine 4, the Bur CDK, and the RNA polymerase II subunit Rpb9. Finally, genetic analysis of sensitivity to NTP-depleting drugs allowed us to find out that the RNA cleavage-factor TFIIS plays an important role during transcription elongation of RP genes. Our work demonstrates that the regulation of transcription elongation is a widespread phenomenon, which affects important gene classes like the RP regulon.

Funded by Grants BFU2007-67575-C03-02 & 01 (Spanish Ministry of Science and Innovation) and **P07-CVI-02623 (Andalusian Government).**

GENE EXPRESSION IS A CIRCULAR SYSTEM.

***Mordechai Choder*[1]**, Gal Haimovich[1], Sebastien Causse[2], Oren Barkai[1], Xavier Darzacq[2]

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The balance between mRNA synthesis and decay determines mRNA levels in the cell. We have recently shown that RNA polymerase II controls mRNA translation[1] and decay[2] that occur in the cytoplasm. We asked whether, conversely, the mRNA decay machinery can impact transcription. We found that maintaining mRNA levels in yeast is highly robust to perturbations in mRNA decay machinery. Defects in a number of cytoplasmic mRNA decay factors lead to down-regulation of transcription. Consistently, these factors shuttle between the cytoplasm and the nucleus, in a manner dependent on proper exonucleolytic degradation of mRNA. In the nucleus, the decay factors are associated with chromatin and stimulate transcription initiation by RNA polymerase II. Our findings demonstrate that gene expression is a circular process in which the hitherto first and last stages are interconnected.

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Funded by the Israel Science Foundation.

Posters

Poster # 1

MICRO-RNAS-10a AND -10b CONTRIBUTE TO RETINOIC ACID-INDUCED DIFFERENTIATION OF NEUROBLASTOMA CELLS AND TARGET THE ALTERNATIVE SPLICING REGULATORY FACTOR SFRS1 (SF2/ASF)

Meseguer, S.[1]*, G. Mudduluru*[2]*, J. M. Escamilla*[1]*, H. Allgayer*[2]*and D. Barettino*[1]

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[2]Dept. of Experimental Surgery/Molecular Oncology of Solid Tumors (Collaborative Unit DKFZ-Heidelberg), Mannheim Medical Faculty, Ruprecht-Karls-University Heidelberg, Mannheim, Germany

MicroRNAs (miRNAs) are an emerging class of non-coding endogenous RNAs involved in multiple cellular processes, including cell differentiation. Treatment with Retinoic Acid (RA) results in neural differentiation of neuroblastoma cells. We wanted to elucidate whether miRNAs contribute to the gene expression changes induced by RA in neuroblastoma cells, and whether miRNA regulation is involved in the transduction of the RA signal. We show here that RA treatment of SH-SY5Y neuroblastoma cells results in profound changes in the expression pattern of miRNAs. Up to 42 different miRNA species significantly changed their expression (26 upregulated and 16 downregulated). Among them, the closely related miR-10a and -10b showed the most prominent expression changes. Induction of miR-10a and -10b by RA also could be detected in LA-N-1 neuroblastoma cells. Loss of function experiments demonstrated that miR-10a and -10b are essential mediators of RA-induced neuroblastoma differentiation and of the associated changes in migration, invasion, and *in vivo* metastasis. In addition, we found that the SR-family splicing factor SFRS1 (SF2/ASF) is a target for miR-10a -and -10b in HeLa and SH-SY5Y neuroblastoma cells. We show here that changes in miR-10a and -10b expression levels may regulate SFRS1-dependent alternative splicing and translational functions. Taken together, our results give support to the idea that miRNA regulation plays a key role in RA-induced neuroblastoma cell differentiation. The discovery of SFRS1 as direct target of miR-10a and -10b support the emerging functional interaction between two post-transcriptional mechanisms, microRNAs and splicing, in the neuronal differentiation context.

Funded by projects SAF2006-00647 and SAF2007-60780 (Ministry of Science and Innovation) and ACOMP 09/212 (Generalitat Valenciana). S. M.was the recipient of an EACR training and travel fellowship award and a CSIC I3P predoctoral fellowship/contract.

Poster # 2

INCREASED PROCESS UNDERSTANDING THROUGH CHARACTERIZATION OF HOST-CELL PROTEIN PATTERNS

A. Hagner McWhirter, S. Grimsby, L. Kask, M. Winkvist,
A. Jorsback, L. Jonsson, T. Björkman, L. Björkesten and G. Malmquist

GE Healthcare Bio-Sciences AB, Uppsala, Sweden

Introduction

Many pharmaceuticals, such as monoclonal antibodies are produced from genetically engineered mammalian cell lines. Purity with regard to undesired host-cell proteins (HCP) of final product is essential for product safety. Process development by varying upstream conditions and analyze the effects on downstream processing is needed for optimal yield and purity. We have characterized HCP patterns in purification of monoclonal antibodies from CHO cells by using 2-D Fluorescence Difference Gel Electrophoresis (2-D DIGE).

Methods

2-D DIGE technology with two different samples and a pooled internal standard per gel pre-labeled with CyDye™ DIGE Fluor minimal Dyes, detects differences in protein abundance. Experimental variation is virtually eliminated and the quantitative data is very reliable. Samples from culture supernatant or MabSelect SuRe™ eluent fractions were buffer exchanged and concentrated into DIGE labeling buffer using Vivaspin columns MwCO 5 kDa. A set of three spiked proteins were added to all samples including the pooled internal standard before CyDye labeling. Protein samples and pooled internal standard were labeled with CyDye minimal DIGE fluors and subjected to 2-D electrophoresis using IPG strips pH 3-11NL and the new pre-cast DIGE Gels followed by scanning using Ettan™ DIGE Imager. Differences in protein levels between samples were analyzed taking advantage of the spike proteins for sample-to-sample normalizaton and DeCyder™ 2D differential analysis software version 7.1.

Results

Differences in protein expression between culture supernatants grown with a set of altered media compositions were analyzed. Also, differences in the HCP patterns of MabSelectSuRe™ eluent fractions were analyzed. The results were related to yield of target protein and HCP levels obtained with ELISA assay.

Poster # 3

HIGH-THROUGHPUT SEQUENCING OF *HOP STUNT VIROID*-DERIVED AND PLANT ENDOGENOUS SMALL RNAS FROM CUCUMBER LEAVES AND PHLOEM

***German Martinez*[1]**, *Livia Donaire*[2], *Cesar Llave*[2], *Vicente Pallas*[1]* *and Gustavo Gomez*[1].

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Viroids are the lowest frontier on biology scale. Their genome consists on a single-stranded RNA, circular, highly structured and without protein coding capacity. One of the potential mechanisms by which viroids are able to trigger a pathogenic signal has been recently related to the production of viroid derived small RNAs (vd-sRNAs). In this work, we have employed massive pyrosequencing techniques (Roche 454 platform) to decipher wich mechanisms and components from the silencing machinery underlie over vd-sRNAs biogenesis of a Hop Stunt Viroid (HSVd) infection on a natural host (Cucumis sativus). HSVd vd-sRNAs characterized were mostly 21- and 22- nucleotides in length and derived equally from plus and minus HSVd-RNA strands. Our sequence data suggest that viroid-derived double-stranded RNA functions as one of the main precursors of vd-sRNAs. Remarkably, phloem vd-sRNAs accumulated preferentially as 22 nt species with a consensus sequence overrepresented suggesting the existence of a selective trafficking of vd-sRNAs to the phloem tissue of infected cucumber plants.

Furthermore, we have also studied the intrinsic sRNA populations on infected and healthy tissues to get deeper on the knowledge of the whole plant post-transciptional behaviour to a viroid infection. Our data show for the first time a high deregulation on miRNAs (miRNAs 159, 167, 171, 397, 398 and 408 are over-represented) and on the levels of the sRNAs derived from the mature ribosomal regions (20-fold increase on specific regions over the ribosome sequence) that occur on a viroid infection and that could be key components on the viroid pathogenesis phenomena.

Poster # 4

ngs_backbone, A SOFTWARE FOR NGS BASED TRANSCRIPTOME ANALYSES, A TOMATO SNP CALLING EXAMPLE

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The possibilities offered by next-generation sequencing platforms are revolutionizing biotechnological laboratories. A field especially impacted by the arrival of these new technologies is that of transcriptomic analysis in non-model species. However, these new sequencing technologies cannot be analyzed with software designed for Sanger sequencing. Besides, the combination of NGS sequencing and affordable high-throughput genotyping technologies is rapidly allowing the discovery and use of SNPs in non-model species. This is abundance of SNPs also creates new software needs. Using a tomato example, this study describes a tool called ngs_backbone that is capable of carrying out these analyses. To test ngs_backbone, a complete analysis of the tomato transcriptome has been done, from the read cleaning to the experimental SNV validation.
All public tomato Sanger EST reads were included in this study. In addition, 14.2 million Illumina reads were also processed. The cleaned reads were mapped to the SGN tomato transcriptome obtaining a coverage of 4.2 for Sanger and 8.5 for Illumina. 33,306 SNVs were found, and after applying several filters to select those with a higher likelihood of being real, a set of 23,360 SNVs was obtained. A total of 76 SNVs of this latter collection were experimentally validated, and at least 85% were found to be real. Finally, a collection of 2,855 highly polymorphic SNVs was created. These collection of SNVs will be a useful resource for tomato researchers and breeders. Moreover the tool presented will facilitate the use of these technologies by experimental laboratories by providing a robust and well-documented software.

Poster # 5

QUANTITATIVE COMPARISON BY MEANS OF FBA OF THE GENOME-SCALE BASED METABOLIC RECONSTRUCTIONS OF Bge and Pam STRAINS OF *BLATTABACTERIUM SP.*

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Blattabacterium strains Bge and Pam are the primary endosymbionts of two species of cockroaches: *Blattella germanica* and *Periplaneta americana*, respectively. Recently, the sequencing of the genomes of these symbionts has been completed [1][2], emerging the opportunity to attempt a reconstruction of a genome-scale computational model of their metabolic processes. *In silico* analyses provide valuable insight into the biology of endosymbionts, their metabolic capabilities and interactions with the hosts. We have reconstructed and analyzed the metabolic network of these bacteria using Flux Balance Analysis (FBA), which represents an effective means to analyze biological networks in a quantitative manner [3]. For our reconstruction, we employed as reference the *i*JR904 model of *Escherichia coli* K-12 [4], eliminating all reactions coded by genes without homology in the mentioned *Blattabacterium* strains or present in them but not connected to the biomass reactions. The metabolic model of *Blattabacterium* str. Bge, named *iBBge10,* accounts for 236 genes and 285 biochemical reactions the encoded proteins carry out (GPRs), whereas *i*BPam10, the model of Pam strain, is composed by a fewer number of genes (229) and gene-protein-reaction associations (282), mainly due to the lack of certain genes encoding enzymes involved in the Krebs cycle and in the sulfur metabolism in the latter strain. We have focused on the differences between both *Blattabacterium* strains to predict their growth rate in a dynamic way and with different conditions (several carbon, nitrogen and energy sources), as a means of simulating a most likely environment where they are living. In addition, as expected, these networks also showed a higher fragility than *E. coli* model, with 70-75% of essential genes for growth. This fact suggests an uniform environment for endosymbiosis.

References:

[1] López-Sánchez, M.J., Neef, A., Peretó, J., Patiño-Navarrete, R., Pignatelli, M., Latorre, A., Moya, A. (2009). Evolutionary convergence and nitrogen metabolism in *Blattabacterium* strain Bge, primary endosymbiont of the cockroach *Blattella germanica*. *PLoS Genet. 5*: e1000721.

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Poster # 6

NEW INSIGHTS OVER THE FUNCTIONAL INTERACTIONS OF THE YEAST HISTONE ACETYLTRANSFERASE Sas3p

***Vicente S.*[1]*, L.Magraner*[1]*, E.Machí*[1]*, L.Valero*[2]*, V.Tordera*[1] *and M.Pamblanco*[1]

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Numerous multiprotein complexes exist within nuclei to modulate the nucleosomal environment, including those with histone acetyltransferase (HAT) activity. Sas3p is the catalytic subunit of the nucleosomal H3-specific HAT complex, NuA3. The loss of *SAS3* results in only very minor phenotypes; however, simultaneous loss of *SAS3* and *GCN5* (the catalytic subunit of several HAT complexes) is synthetically lethal. Both HATs acetylate H3K14 and are recruited to a similar pool of intensively transcribed genes. Here, by ChIP-chip analyses using tiled microarrays, we found that Sas3p is associated to the coding regions of genes showing a peak enrichment within the 5' half of the ORF that drops substantially towards the 3' region of the ORF. This unexpected result suggests the involvement of NuA3 in transcriptional elongation. To further analyze the redundant function of Sas3p and Gcn5p in the acetylation state of H3K14 *in vivo,* we have purified and characterized, by the TAP-MS (Tandem affinity purification-mass spectrometry) strategy, multiprotein complexes associated to Sas3 from TAP strains deleted or not in *GCN5* gene. Both native purifications present HAT activity. Interestingly, together with the well-established components of NuA3 (Nto1, Taf14, Yng1 and Eaf6), all the four nucleosomal histones and Ylr455w, a functionally unknown protein containing a DNA-interacting motif, also copurify with Sas3p.

Poster # 7

ANALYSIS OF THE EXCRETORY/SECRETORY PROTEOME OF THE ADULT STAGE OF *Echinostoma caproni*

***Sotillo J.*[1]*, Cortés A.*[1]*, Trelis M.*[1]*, Valero, M.L.*[2]*, Sánchez del Pino M.M.*[2]*, Marcilla A.*[1]*, Esteban J.G.*[1] *and Toledo R.*[1]

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[2] Laboratorio de Proteómica, Centro de Investigación Principe Felipe, Valencia, Spain.

The excretory/secretory proteome of the adult stage of *Echinostoma caproni* has been analyzed using a shot-gun LC/MS-MS for the separation and identification of tryptic peptides. MASCOT search engine (Matrix-Science) and ProteinPilot software v2.0 (Applied-Biosystems) were used to search homologies in the databases, and a total of 39 proteins were accurately identified. Strikingly, metabolic enzymes, and particularly glycolytic enzymes, constituted the largest protein family in the excretory/secretory proteome of *E. caproni* adult worms. Moreover, proteins involved in parasite structure, response against stress, chaperones, calcium-binding and signal transduction were also identified. Although the low number of sequences of echinostomes deposited significantly limits the identification of a greater number of proteins, the present study will facilitate the use of echinostomes as experimental models, which may be of great importance to gain further insight in the host-parasite relationships in intestinal helminth infections.

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Poster # 8

QUANTIFYING THE RELATIONSHIP BETWEEN SEQUENCE AND THREE-DIMENSIONAL STRUCTURE CONSERVATION IN RNA

Emidio Capriotti and ***Marc A Marti-Renom***

Structural Genomics Unit, Bioinformatics and Genomics Department, Centro de Investigación Príncipe Felipe, Valencia, Spain

Background: In recent years, the number of available RNA structures has rapidly grown reflecting the increased interest on RNA biology. Similarly to the studies carried out two decades ago for proteins, which gave the fundamental grounds for developing comparative protein structure prediction methods, we are now able to quantify the relationship between sequence and structure conservation in RNA.

Results: Here we introduce an all-against-all sequence- and three-dimensional (3D) structure-based comparison of a representative set of RNA structures, which have allowed us to quantitatively confirm that: (i) there is a measurable relationship between sequence and structure conservation that weakens for alignments resulting in below 60% sequence identity, (ii) evolution tends to conserve more RNA structure than sequence, and (iii) there is a twilight zone for RNA homology detection.

Discussion: The computational analysis here presented quantitatively describes the relationship between sequence and structure for RNA molecules and defines a twilight zone region for detecting RNA homology. Our work could represent the theoretical basis and limitations for future developments in comparative RNA 3D structure prediction..

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Poster # 9

UN NOVEDOSO SISTEMA DE "TRES COMPONENTES" REGULA LA RESPUESTA A ESTRÉS EN CIANOBACTERIAS

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En microorganismos los sistemas de transducción de señal más importantes, conocidos como de dos componentes (TCS), implican la autofosforilación de una histidina quinasa (HK) en un residuo de histidina conservado y la posterior transferencia del grupo fosforilo a un residuo de aspártico de un regulador de la respuesta (RR). En relación al tamaño de sus genomas, las cianobacterias son los microorganismos con un número mayor de genes que codifican para TCS. En estos organismos, NblS es la HK más conservada, jugando un papel clave en la regulación de la fotosíntesis y la aclimatación a una gran variedad de condiciones ambientales. A pesar de la importancia de esta HK, su pareja para formar un TCS es todavía desconocida. Utilizando diferentes aproximaciones *in vivo*, *in vitro* e *in silico* hemos identificado a RpaB y SrrA como los reguladores de la respuesta socios de NblS. Nuestros análisis genéticos demuestran que RpaB y NblS son esenciales en *Synechococcus elongatus* PCC 7942, y que la fosforilación de ambos es también necesaria para la viabilidad de este organismo. Por el contrario, SrrA no es esencial, pero nuestros ensayos *in vitro*, indican que NblS muestra una fuerte preferencia por SrrA frente a RpaB en la reacción de fosforilación. Esta aparente discrepancia puede ser explicada por el aislamiento de ambas rutas, que se consigue vía la represión de *srrA* por parte de RpaB durante condiciones propicias de crecimiento. La exposición a estrés produce un fuerte y transitorio incremento en la expresión de *srrA*. La posterior represión de *srrA* y otros genes de respuesta a estrés, sugiere que existe una cooperación entre RpaB y SrrA. Estos datos muestran por primera vez un sofisticado circuito regulatorio donde intervienen dos RR ortodoxos controlados por una misma HK, aportando un paradigma para regulaciones múltiples en TCS ramificados.

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Poster # 10

MIP6, A NEW COMPONENT OF THE RNA EXPORT MACHINERY BINDS TO SPECIFIC mRNAS ENCODING FUNCTIONALLY RELATED PROTEINS.

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In eukaryotes, prior to translation, mRNAs need to be exported from the nucleus to the cytoplasm trough the NPC. In this process from transcription to translation, mRNAs interact with different factors that play important roles at different levels of RNA protection, export and surveillance.
The widely characterized protein Sus1 is a key factor in this process and it has been shown that this factor couples gene regulation and mRNA export. Here we present new data regarding the protein Mip6, an uncharacterized member of the Sus1 pathway involved in mRNA export. Mip6 is an RNA binding protein that physically interacts with Sus1 and also with the heterodimeric export receptor Mex67/Mtr2 among others. Under heat shock conditions Mip6 forms cytoplasmatic structures resembling stress granules and we have found that in this circumstances, far from carrying a general pool of RNAs, Mip6 is associated to specifics RNAs encoding mostly components of proteolysis machinery. We present Mip6 as a novel RNA binding protein and as component of specific heat shock mRNPs involved in the export of mRNAs encoding functionally related proteins that may contribute to cell survival under stress conditions.

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Poster # 11

LA SECCIÓN DE CHIPS DE DNA del SERVEI CENTRAL DE SUPOR A LA INVESTIGACIÓ EXPERIMENTAL (SCSIE) de la UNIVERSITAT DE VALÈNCIA

José García-Martínez.

Secció de Xips de DNA. S.C.S.I.E. Universitat de València

En esta Sección, integrada en el Servei Central de Suport a la Investigació de la Universitat de València, se desarrolla la tecnología de arrays de DNA tanto en su versión de macro- (sobre nylon) como de microchips (sobre vidrio). Para ello se dispone de la tecnología para la fabricación de Macrochips (filtros) de DNA, basada en la utilización de productos de PCR específicos de cada gen que se fijan sobre filtros de Nylon mediante un sistema robotizado a una densidad de 75 muestras/cm2 y permitiendo analizar el genoma completo de un organismo o un número muy elevado de sus genes en un solo experimento. Debido a que la detección de las señales en los filtros de nylon se debe hacer mediante marcaje radioactivo, también se dispone del correspondiente phosphorimager para captar dichas señales i generar imágenes de las hibridaciones.
Análogamente se dispone también de un robot para la fabricación de "Microseries" (microchips) en soporte de vidrio, con la consiguiente mayor densidad de impresión y mayor número de genes analizados simultáneamente. En este caso debido a que el marcaje es fluorescente, se dispone de un scanner con 2 láseres que permite el análisis de 2 muestras simultáneas hibridadas sobre el mismo array.
En ambos casos se dispone del software correspondiente para analizar las imágenes de hibridación generadas y poder cuantificar las intensidades de cada uno de los spots tras la hibridación.

En breve se va a disponer de un nuevo equipamiento de secuenciación masiva que permitirá igualmente realizar estudios de expresión a nivel genómico.

En cuanto a los servicios que se ofertan serían los siguientes:

- Comercialización del macrochip de genoma completo de levadura.
- Impresión de chips “a la carta”, tanto en la versión macro como micro, a partir de las sondas de usuario, o mediante la obtención de dichas sondas por el procio servicio.
- Procesado experimental de de los chips, tanto marcaje radioactivo para macroarrays como fluorescente para microarrays. Estos últimos, tambien los microarrays comerciales para cualquier organismo.
- Análisis estadísticos de los resultados, y entrega de resultados finales de la comparación entre condiciones.
- Análisis de condiciones múltiples, y agrupamientos de perfiles (clustering).

Poster # 12

SEM1 IS REQUIRED FOR TREX2 STABILITY AND SUS1 ASSOCIATION TO SAGA

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Sem1 is a conserved component of the lid subcomplex of the 19S regulatory particle of the 26S proteasome. Several studies have established links between components of the mRNA export machinery and Sem1 in a proteasome-independent manner. Here, we provide additional data that support this functional and physical association and new evidences that show a role for Sem1 along transcription. We show through biochemical analyses that, Sem1 and Sus1, a bona fide component of TREX2 and SAGA complexes, interact physically. This interaction occurs in the context of TREX2, since Sac3 and Thp1 are necessary. However, Sus1 is dispensable for Sem1 binding to TREX2. Moreover, Sus1 and Sem1 partially overlap in their functions in transcription and mRNA export. This last process is significantly affected in the double mutant sus1Δsem1Δ. Strinkly, we observe that Sus1 is preferentially associated with SLIK and histones in absence of SEM1. Therefore, Sem1 is required for the dynamic interactions of Sus1 with both complexes SAGA and SLIK. Finally, we show that Sem1 is required for regular Sus1 cellular distribution. Here we present new data supporting that the proteasomal subunit Sem1 is required in a proteasome-independent fashion for the coordinated function of SAGA and TREX2 mediated by Sus1.

Poster # 13

STUDY OF *Saccharomyces cerevisiae* RESPONSE TO DIFFERENT ANTIMICROBIAL PEPTIDES USING A GENOMIC APPROACH.

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The use of antimicrobial peptides (AMP) as alternative to the control of phytopathogenic fungi has been proposed and demonstrated by several groups[1]. Recent works suggest that AMP mode of action is a complex process that can involve specific interactions and intracellular mechanisms. PAF26 is a synthetic antimicrobial hexapeptide capable to translocate inside cells as demonstrated in filamentous fungi and *Saccharomyces cerevisiae*[2,3]. The aim of this study is the characterization of the antifungal effect of PAF26 compared to melittin, a natural lytic peptide, using genomic tools and the yeast *S. cerevisiae* as model organism. We analyzed the transcriptome changes in *S. cerevisiae* after exposure to sub-inhibitory peptide concentrations. Hybridization of macroarrays containing the yeast genome had shown a differential effect of either PAF26 or melittin, supporting a distinct mode of action. Composition and structure of cell wall was the only process induced by both peptides, while differential expression response included genes related to ribosomal biogenesis and rRNA processing, metabolism of arginine and amino groups (*ARG* genes), or HSP proteins involved in protein folding during stress. Assays carried out with deletants in cell wall-related genes only showed genes having limited influence on sensitivity to peptides. Flow citometry studies and fluorescence microscopy showed that PAF26 cell interaction correlated with cell death in cell wall mutants. Deletants in *ARG* genes showed increased resistance to PAF26 but bound peptide as the parental strain. These results suggest that induction of genes related to cell wall is a general response to exposure of *S. cerevisiae* to distinct AMP with different properties. Cellular permeation is not primary mode of action of PAF26 and intracellular processes as arginine metabolism are likely involved in susceptibility to peptides.

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Poster # 14

UNDERSTANDING MOLECULAR BASIS OF RIPENING IN CITRUS FRUIT: INVOLVEMENT OF ABA

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'Pinalate' orange is an abscisic acid (ABA) deficient spontaneous mutant from the 'Navelate' orange (*Citrus sinensis* L. Osbeck). Fruits from this mutant cannot achieve the characteristic color of its parental and remain yellow because of an altered carotenoid composition along ripening. Therefore, 'Pinalate' fruit is an excellent experimental system to perform transcriptomic studies aimed to understand molecular basis of citrus fruit ripening and to analyze the potential role of ABA in this process. In this work, global changes in gene expression occurring in the outer part of the peel (flavedo) from 'Navelate' and 'Pinalate' fruits have been studied along three ripening stages: Mature green (MG), breaker (Bk) and full colored (FC). Color index in parental FC fruits was 6-fold higher than in mutant fruit, which showed similar color to those of the parental Bk fruits. Transcriptomic analysis performed by using a cDNA microarray containing about 21000 unigenes, generated in the Spanish 'Citrus Functional Genomic Project' (CFGP), revealed that major changes in gene expression occurred during the transition from MG to Bk stage. The number of induced genes (1210) in Bk respect to MG fruits was about 4-fold higher in 'Pinalate' than in its parental (278), while the number of repressed genes was similar in both genotypes. Gene ontology analysis revealed that biological processes related to photosynthesis were inhibited in both 'Navelate' and 'Pinalate' fruits. Other processes such as glycerolipid metabolism and flavonoid and oxylipin biosynthesis were down-regulated only in the mutant, while response to water deprivation and glucan biosynthetic processes were only over-represented in parental fruits.

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Poster # 15

SERVICIO DE SECUENCIACIÓN DE DNA, GENOTIPADO Y ANÁLISIS DE MARCADORES MOLECULARES.

***Unidad de Genómica**-Servicio central de apoyo a la investigación experimental. Universitat de València.*

Edificio de Investigación Jeroni Muñoz. Lab. 0-49. C/ Dr. Moliner 50. Burjassot -4610, Valencia. (secuenciacion@uv.es)

El Laboratorio de Secuenciación de DNA, Genotipado y Análisis de Marcadores Moleculares forma parte de la Unidad de Genómica del Servicio Central de Soporte a la Investigación Experimental de la Universitat de València. En nuestro laboratorio se desarrollan protocolos y proyectos basados en diferentes técnicas de Genética y Biología Molecular que se pueden agrupar en las siguientes áreas:

- Secuenciación automática de DNA mediante electroforesis capilar
- Genotipado de diversos marcadores de DNA:
    - análisis cualitativos de RFLPs, STRs o microsatélites, SNPs
    - análisis semicuantitativos como estudios de MLPA y CNVs
- Determinación y análisis de la variación genética de marcadores moleculares aplicados al estudio de especies determinadas.
- Estudios de expresión génica mediante PCR en tiempo real

Además, en el SCSIE se pretende proporcionar acceso a las tecnologías más innovadoras, con lo que se van a poner a disposición de todos los grupos de investigación de la Universitat de València u otros centros públicos o privados, dos equipos de secuenciación masiva, la plataforma SOLID-4 (Applied Biosystems) y el equipo GS Junior (Roche). Dadas las características de estos equipos, se podrán desarrollar distintas aplicaciones como:

- secuenciación de transcriptomas completos: expresión de todos los RNAs codificantes y no codificantes, identificación de “splicing” alternativo, identificación de patrones de expresión específicos de alelo.
- secuenciación de exomas: enriquecimiento de exoma completo con la tecnología de Agilent “whole exome sure select”.
- secuenciación *de novo* de genomas microbianos.
- secuenciación de amplicones (producto de PCR).

Poster # 16

STRUCTURAL BIOLOGY OF VIRUS: PHAGE THERAPY

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Bacteriophage T4 recognizes its host cells *via* its long tail fibre glycoprotein (gp)37. The gp37 contains 1026 amino acids per monomer forming a fibrous parallel homotrimer at the distal end of the long tail fibres. The other distal half-fibre protein gp36 is much smaller forming a trimer of 221 amino acids per monomer. Functional and structural studies of gp37 were hampered by the inability to produce suitable amounts of it. Soluble gp37 was produced by co-expression with two T4-encoded chaperones in a two-vector system; co-expression with each chaperone separately did not lead to good amounts of correctly folded trimeric protein. An expression vector for T4 fibrous protein chaperone gp57 was co-transformed into bacteria, with a compatible bi-cistronic expression vector containing T4 genes 37–38. A six-histidine tag was encoded amino-terminal to gene gp37. Recombinant trimeric gp37, containing the histidine tag and residues 12–1026 of gp37, was purified from lysed bacteria by subsequent Ni-affinity, size-exclusion and strong anion exchange column chromatography. Yields of *ca*. 4mg of purified protein per litre of bacterial culture were achieved. Electron microscopy confirmed the protein to form fibres *ca*. 63nm long; presumably gp36 makes up the remaining 11nm in the intact distal half-fibre. Purified, correctly folded gp37 is useful for receptor-binding and high-resolution structural studies and specific binding and detection of bacteria. A promising and emerging area of research in aquatic environments is the application of *phage therapy* for disease management. Hankin published in 1896 the first report that has to do with phage therapy: the bactericidal action of the Water of the Jumma and of Ganges on the vibrio of cholera. Though the *phage* concept was discovered in 1914, the first reported application of phage therapy was by Bruynoghe and Maisin in 1921 for the management of *Staphylococcus* skin disease. Phage therapy in aquaculture was first attempted by Wu and Chao in 1982 for Edwardsiellosis in fish. Nakai *et al*. reported the protective effects of bacteriophage on experimental *Lactococcus garvieae* infection in yellowtail. Mohanty and Sahoo reviewed Edwardsiellosis in fish. Almeida *et al*. examined phage and photodynamic therapies as approaches of low environmental impact to inactivate microorganisms in fish farming plants. Some preliminary attempts of phage therapy in aquaculture were reported by the Marine Biotechnology Centre of Excellence of College of Fisheries at Mangalore (India). There seems to an immense scope of further research into the facet of microbial intervention in aquatic environment. One of the major advantages of the method is that the phages are effective and specific to the targeted pathogen unlike the antibiotics, which produce resistance among bacterial species, thus, near elimination of targeted specific pathogens can be achieved. In earlier publications, the exact structure of bacteriophage T4 and infection mechanism were analyzed with the application to stop *Escherichia coli*, the use of T4 for recognition and infection in *E. coli* was examined and the structural biology of the virus and bacteriophage fibre proteins were studied.

Poster # 17

MEASURING THE FUNCTIONAL SIMILARITY OF DRUGS

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We presents a novel strategy to measure the functional similarity of Gene Ontology profiles, applied it to a large dataset of drug-expression-data and compared all profiles against each other to identify similar functional characteristics among them.

Motivation:
As functional profiling/enrichment methods become the standard procedure for interpreting functional genomic studies it becomes more and more necessary to develop bioinformatic methodologies that establish connections within the functional profiling world. We presents a novel strategy to assessing the functional similarity of profiles based on the GO topology. We applied this strategy to an extensive dataset of drug profiles derived from genome-wide expression data. The dominant biological functions of drugs were compared to identify and detect similar functional characteristics among them.

Material and methods:
Drug expression data from the Connectivity Map project was analysed by 2 GSEA methods. The resulting functional profile of 1500 drugs were compared against each other by a 3-step approach: Pairwise “node-based” semantic similarity for each pair of GO terms, the “best-match average” to combine the similarity of profiles and a random based normalization to account for the profile's size bias. The resulting matrix of drug similarities was evaluated by comparing it against the ATC drug classification scheme to show that similar compounds obtained high semantic similarity scores.

Results:
The strategy, based on an edge-based metric to considers the topology of the GO hierarchy was used to measure the functional distances of drug based on a large set of transcriptomics data. A classification scheme for these compounds made it possible to contrast our results with an external criterion of similarity. The ATC system codifies drugs at different levels of specificity and we confirmed that the proposed method captures this specificity and observed that the mean similarity value increased when compared drugs belong to more specific ATC level.

Discussion:
The proposed strategy to assess the semantic similarity of functional profiles combines a edge-based similarity metric with random null-model comparison to assess absolute distances of functional profiles. The analysis includes the evaluation and preprocessing of a large gene-expression drug dataset. To evaluate the functional similarity, drugs had been grouped by the hierarchical ATC classification scheme. The strategy proposed for measuring the semantic similarity of functional profiles has proved to be a valid methodology to study functional proximity between biological samples

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Poster # 18

PROFILING THE VENOM GLAND TRANSCRIPTOMES OF COSTA RICAN SNAKES BY 454 PYROSEQUENCING

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Central American herpetofauna includes 34 species of venomous crotalid snakes. Venom represents a trophic adaptation confering a selective advantage to the snake for the success in the colonization of, and adaptation to, novel hunting territories. However, in regions of sympatry with humans snakebites also represent a relevant, albeit neglected, public health issue. Only in Central America, 5000 cases of snakebite envenomation occur every year. Application of our snake venomics protocols has provided data on the number and distribution of protein families present in the venoms of a number of Costa Rican snake species. To complement and extend this information, we have employed the 454 high-throughput technology (FLX System, Roche Applied Science) to investigate the transcriptional activity of the venom glands of *Bothriechis lateralis*, *Bothriechis schlegelii*, *Atropoides mexicanus*, *Atropoides picadoi*, *Crotalus simus*, *Cerrophidium godmani*, and *Bothrops asper* (Caribbean and Pacific variants). Because of the low data compression gained in the assembly step, the lack of a reference genome and the small difference between contigs and reads mean length, bioinformatic processing of the 454 sequence data was performed on whole sets of unassembled reads. To this end, the set of 330,010 nucleotide reads was searched against the non-redundant NCBI database and "best-hits" for entries of snake proteins were identified. The descriptors were analyzed and grouped into 20 documented snake venom protein families. For each species transcriptome, the number of reads for each venom protein family was calculated, generating a profile of relative abundances of the different families. This expression profile allowed us to group the 8 snake species in kinship groups. Besides, a representative full-length amino acid sequences from a phylogenetically closer species were used as template for the relative alignment of the tblastn hits, thus generating consensus sequences. The structural diversity within each gene family in each species as well as an estimation of the mininum number of genes per protein were also addressed.

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Poster # 19

^{1}H NMR METABONOMICS SERUM BLOOD PROFILING OF MINIMAL HEPATIC ENCEPHALOPATHY

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Minimal Hepatic Encephalopathy (MHE) is a preclinical stage of Hepatic Encephalopathy (HE). HE is a degenerative disease that is induced by malfunctioning liver and causes, among other disturbances, alterations in intellectual function, conscience and motor function and coordination. There is no definitive cure for the disease, but early diagnosis and treatment of MHE improves greatly patient quality of life.
^{1}H NMR spectroscopy enables the simultaneous detection, quantitation and characterisation of multiple metabolites and small molecules in a variety of biological samples (e.g. biofluids, cells and tissues). Collectively, this approach can be referred to as metabonomics.
We have utilised ^{1}H NMR based metabonomics to study the serum metabolic profile of alcohol induced cirrhotic patients with or without MHE. Numerous blood metabolites with differing concentrations between patient groups were identified. MHE patients displayed increased serum concentrations of glucose, lactate and glycerol, as well as decreased levels of choline, alanine and lipid moieties. This approach offers a new objective method of MHE patient classification and may facilitate the study of disease progression.

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Poster # 20

EXPRESSION IN HUMAN CELLS OF RTS-DISINTEGRIN JERDOSTATIN AS AN ACTIVE $\alpha_1\beta_1$ INHIBITOR

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Jerdostatin represents a novel RTS short disintegrin which was recombinantly expressed in *E.coli* from a *Protobothrops jerdonii* cDNA library. Cell adhesion studies showed that was able to block specifically the adhesion of $\alpha_1\beta_1$-expressing K562 cells to immobilized collagen IV. $\alpha_1\beta_1$ is highly expressed on microvascular endothelial cells, and blocking of its adhesive properties significantly reduces the vascularization ratio and tumour growth in mice. The generation of conditioned transgenic mice for jerdostatin will allow the investigation of the effect of targeting the $\alpha_1\beta_1$ integrin during mouse development/growth and in the adult animal. This animal model will be fundamental for evaluating the effect of the continuous expression of jerdostatin upon tumour development in transgenic versus wild-type mice. In order to achieve this objective, an expression plasmid was developed; pRc-CMV/FLAGrJerd was transient transfected into HEK-293T cells. Recombinant jerdostatin (rJerd) was purified by affinity chromatography and detected by Western Blot analysis. To investigate the molecular interaction between rJerd and the $\alpha_1\beta_1$ integrin, we used a soluble heterodimeric $\alpha_1\beta_1$ integrin that binds with high affinity to collagen type IV, similar to the wild-type integrin. As described previously in other non-RGD disintegrins, this interaction does not depend on divalent cations. rJerd was also able to inhibit $\alpha_1\beta_1$ integrin binding to collagen IV (CB3) in a dose-dependent manner (IC_{50} 570 nM). To investigate the biological role of rJerd binding to the $\alpha_1\beta_1$ receptor we carried out cell-adhesion assays with Rugli cells. In the presence of rJerd we observed a blockade the interaction of $\alpha_1\beta_1$ integrins present at the cells with collagen IV, preventing cellular adhesion. In conclusion, all the experiments corroborate that the first recombinant disintegrin expressed in a mammal cell system is active, recognize $\alpha_1\beta_1$ receptor, it's able to inhibit the binding of the integrin to its natural ligand (Collagen IV) and also *in vitro* disrupts Rugli cells' focal adhesions unabling cell adhesion.

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Poster # 21

CRIO4, A GENE FROM SUGAR BEET IS ABLE TO CONFER TOLERANCE TO COLD STRESS BY OVEREXPRESSION

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Abiotic stress is one the main limiting factors for agricultural yield. There are not extensive descriptions on which molecular processes are compromised by suboptimal temperatures. Aiming at identifying plant genes related to cold tolerance we constructed a cDNA library of sugar beet (*Beta vulgaris*) in a yeast expression vector. *CRIO4* was isolated by its ability to form colonies at 10ºC. This gene is conserved in plants and presents several functional domains. Among them, a SEC14 domain. This domain is similar to the *SEC14* gene of yeast (*Saccharomyces cerevisae*). *SEC14* encodes a Phosphatidylinositol/Phosphatidylcholine transfer protein involved in the coordinate regulation of PtdIns and PtdCho metabolism. Our results indicate that lipid transfer activity should be conserved, given that our sequence data shows a conservation of the key aminoacids identified in other organisms. Aiming at confirm this observation we have purified the CRIO4 protein and performed in vitro interaction assays with phospholipids. In addition our study has identified a GOLD domain (related to protein-protein interaction in the Golgi), a Poliproline domain and several PXXP domains. *CRIO4* is homologue to the *PATELLIN1-6* gene family of *Arabidopsis thaliana*. We have identified the members of this family which exhibit higher conservation to *CRIO4*, isolated homozygotic mutants, and we crossed them to obtain double mutants. Phenotypic analysis of those double mutants, as well as localization data of CRIO4 are presented.

This work was funded by grant BFU2008-00604 of the spanish MICINN.

Poster # 22

THE GENOME OF *BLATTABACTERIUM* SP., OBLIGATORY ENDOSYMBIONT OF THE XENOPHAGEOUS WOOD ROACH *CRYPTOCERCUS PUNCTULATUS*

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Cockroaches harbour endosymbionts in their fat body, classified into the genus *Blattabacterium*, that are involved in nitrogen excretion and recycling (1). Three complete genomes from blattabacteria of urban cockroaches are already known while there is few information on the symbionts of other lineages of cockroaches. Wood cockroaches of the family *Cryptocercidae* live in soil and wood logs and exhibit life style similarities with termites like the feeding on dead wood that is digested by an intestinal flora of protozoa. Here we sequence the genome of the symbiont of *Cryptocercus punctulatus*, a species living in North America. Bacterial DNA was sequenced using the 454 technology and assembled with MIRA 2.9. and the Staden package. A single scaffold of 14 contigs representing the whole chromosome of 605 +/- 2 kb as well as a plasmid of 3.6 kb could be assembled yet. The chromosome is thus approximately 35 kb smaller than the three reference blattabacterial genomes and contains 586 functional genes (621 to 627 for the other genomes). With a G+C% of 23.9 the genome is slightly richer in A+T (references 27.1 – 28.2). Chromosome and plasmid show a very high degree of gene synteny with the other three genomes. Among the gene losses are an argininosuccinate lyase (argH), one of the four enzymes constituting the urea cycle, and most genes for the biosynthesis of tryptophan, threonine/isoleucine, and leucine. In summary, the genome of the *C. punctulatus* symbiont constitutes a reduced and further deteriorated version of the *Blattabacterium* sp. genome found in urban cockroach symbionts.

(1) López-Sánchez M.J., A. Neef, J. Peretó, R. Patiño-Navarrete, M. Pignatelli, A. Latorre and A. Moya. Evolutionary convergence and nitrogen metabolism in *Blatttabacterium* strain Bge, primary endosymbiont of the cockroach *Blattella germanica*. PLoS Genetics. 5:e1000721. (2009)

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Poster # 23

HIGH PERFORMANCE AT HIGHEST SPEEDS FOR PROTEIN IDENTIFICATION IN COMPLEX MATRICES

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Abstract: Protein research and protein biomarker discovery can be a time-consuming undertaking due to the complexity of biological samples. To deeply interrogate a sample, instrument sensitivity is critical. However with MS instruments available today high speed MS/MS acquisition is performed at the expense of high quality data. This is detrimental as higher mass accuracy and higher resolution improves the specificity of peptide identification. In this research the TripleTOF™ 5600 system capable of achieving high acquisition speeds while maintaining high performance was investigated to understand the advantages for protein identification from complex samples.

In this presentation complex cell lysates were denatured, reduced and aklyated and digested with trypsin. The sample was analyzed using the Eksigent nanoLC-Ultra™ 2D System combined with the cHiPLC™-Nanoflex system in Trap-Elute mode. The eluent was analyzed using the TripleTOF™ 5600 system with the NanoSpray® Source and heated interface. TOF MS survey scans (>30000 resolution) were collected and 10-50 MS/MS scans (at >15000 resolution) in a second were acquired. Various sample loadings on column were run to explore the sensitivity and speed of the instrument for in-depth sample interrogation. Each dataset was searched with ProteinPilot™ Software 4.0 using the Paragon™ Algorithm in thorough mode with biological modifications. False discovery rate (FDR) analysis was done by on-the-fly analysis of the reversed sequences using the embedded PSPEP tool.

Preliminary identification results indicate that significant improvements in the number of proteins and peptides identified in a single acquisition have been achieved through the increase in sensitivity, speed and resolution of the new instrument. Even at a lower loading of 20 ng of *Ecoli* cell lysate, very large numbers of peptide identifications were observed, 6157 peptides and 729 proteins. The mass accuracy observed was very good, RMS error of 1.72 for the whole dataset. Peptides were detected with precursor signal across 4 orders of dynamic range.

Poster # 24

INCREASED PROCESS UNDERSTANDING THROUGH CHARACTERIZATION OF HOST-CELL PROTEIN PATTERNS

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Introduction

Many pharmaceuticals, such as monoclonal antibodies are produced from genetically engineered mammalian cell lines. Purity with regard to undesired host-cell proteins (HCP) of final product is essential for product safety. Process development by varying upstream conditions and analyze the effects on downstream processing is needed for optimal yield and purity. We have characterized HCP patterns in purification of monoclonal antibodies from CHO cells by using 2-D Fluorescence Difference Gel Electrophoresis (2-D DIGE).

Methods

2-D DIGE technology with two different samples and a pooled internal standard per gel pre-labelled with CyDye™ DIGE Fluor minimal Dyes, detects differences in protein abundance. Experimental variation is virtually eliminated and the quantitative data is very reliable. Samples from culture supernatant or MabSelect SuRe™ eluent fractions were buffer exchanged and concentrated into DIGE labeling buffer using Vivaspin columns MwCO 5 kDa. A set of three spiked proteins were added to all samples including the pooled internal standard before CyDye labeling. Protein samples and pooled internal standard were labelled with CyDye minimal DIGE fluors and subjected to 2-D electrophoresis using IPG strips pH 3-11NL and the new pre-cast DIGE Gels followed by scanning using Ettan™ DIGE Imager. Differences in protein levels between samples were analyzed taking advantage of the spike proteins for sample-to-sample normalization and DeCyder™ 2D differential analysis software version 7.1.

Results

Differences in protein expression between culture supernatants grown with a set of altered media compositions were analyzed. Also, differences in the HCP patterns of MabSelectSuRe™ eluent fractions were analyzed. The results were related to yield of target protein and HCP levels obtained with ELISA assay.

Poster # 25

SNAKE VENOMICS OF THE RATTLESNAKE *Crotalus durissus cascavella FROM BRAZIL*

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Venom proteins from the rattlesnake *Crotalus durissus cascavella* were separated by reverse-phase HPLC using a C18 column, and N-terminal sequencing (using a Procise instrument, Applied Biosystems, Foster City, CA, USA), MALDI-TOF mass fingerprinting and CID-MS/MS were determined. The relative abundances (% of the total venom proteins) of the different protein families in the venom were estimated, and the relative contributions of different proteins eluting in the same chromatographic fraction were estimated by densitometry after SDS-PAGE separation. Amino acid sequence similarity searches were performed against the available databanks using the BLAST program implemented in the WU-BLAST2 search engine at http://www.bork.embl-heidelberg.de. The venom proteome of *C. d. cascavella* comprises 20-25 main toxins in the range of 4-115 kDa and belonging to the following 8 toxin families: disintegrin, PLA_2, serine proteinase, cysteine-rich secretory protein (CRISP), vascular endothelial growth factor-like (VEGF), L-amino acid oxidase, C-type lectin-like, and snake venom metalloproteinase (SVMP). Venom is predominantly composed of neurotoxic heterodimeric phospholipase crotoxin (72.5%), followed by a myotoxic PLA_2 (18,1%). These toxins are responsible for the main clinical manifestations observed by *C. durissus* envenomations: systemic neurotoxicity and myalgic symptoms and coagulation disturbances, frequently accompanied by myoglobinuria and acute renal failure.

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Poster # 26

SEARCH FOR THE GENE RESPONSIBLE FOR A NEW CLINICAL FORM OF HEREDITARY RECURRENT NEUROPATHY

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Inherited peripheral neuropathies that are recurrent and from which affected individuals make full or partial degrees of recovery are unusual. The most prominent disorders that fall into this category are: (i) hereditary neuropathy with liability to pressure palsies (HNPP; MIM 162500) caused by mutations in the *PMP22* gene; (ii) hereditary neuralgic amyotrophy (HNA; MIM 162100) due to mutations in the *SEPT9* gene; and (iii) primary erythromelalgia (MIM 133020) caused by mutations in the *SCN9A* gene. We recruited a family whose proband had the inferred diagnosis of recurrent hereditary neuropathy. With the aim to characterize the molecular bases which underlie this neuropathy, we first analyzed the candidate genes/loci (*PMP22*, *SEPT9* and *SCN9A*) by automated sequencing or by segregation analysis. Findings showed that none of these three genes are involved in the disease. Next, we carried out a genome-wide analysis (Affymetrix GeneChip® Human Mapping 500k Array Set) and the subsequent linkage analysis using the Merlin software allowed us to identify a 12.39 Mb region located on chromosome 21 (Z_{max}> 3, θ= 0.00). This mapped region contains 20 protein and 9 miRNA coding genes and includes interesting functional candidate genes, such as *APP (Amyloid Precursor Protein)*, *NCAM2* (*Neural Cell Adhesion Molecule 2*) or *GABPA* (*GA Binding Protein transcription factor, alpha subunit*). Mutations in exons of these 29 coding genes and their intronic flanking regions were discarded by automated sequencing. We have also discarded the possible existence of deletions and/or duplications on this region by a Human Custom CGH Microarray 4x44K (Agilent p/n G4426A-29618). To date the disease causing mutation of this novel inherited peripheral neuropathy remains unknown.

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Poster # 27

COMBINING EXPRESSION PROFILING AND ChIP-on-chip ANALYSIS TO IDENTIFY PUTATIVE TARGETS OF Cabut DURING DORSAL CLOSURE IN *D. melanogaster*

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Dorsal closure (DC) is a morphogenetic movement in which the lateral epithermal sheets migrate and fuse over the amnioserosa to form a continuous larval epithelium. There are several genes involved in this process, one of them being *cabut* (*cbt*) that encodes a C_2H_2 zinc finger transcription factor. *cbt* mutant embryos present an anterior hole and show defects in the elongation of the dorsal-most epidermal cells as well as in the actomyosin cable assembly at the leading edge. We previously demonstrated that Cbt is required downstream of the JNK pathway during DC, regulating *dpp* expression in the leading edge cells. In order to identify genes functionally related to Cbt during DC (stage 10-13), we have used Genome 2.0 Affymetrix DNA microarrays to compare the expression profiles of *wild-type* and *cbt* mutant embryos. We have identified a set of approximately 1000 genes which are either positively or negatively regulated by Cbt with a fold change of at least 1.5. We find that some of them are involved in JNK signaling, cytoskeleton dynamics, and ecdysone/insulin response. To validate these results, we have used quantitative real-time PCR, genetic and in vivo expression analyses. Furthermore, we have recently performed chromatin immunoprecipitation assays combined with microarray analyses (ChIP-on-chip), in collaboration with the modENCODE project, to identify direct Cbt targets. Our preliminary results show that Cbt binds to genomic regions close to ~180 genes whose expression is probably regulated by this protein. Interestingly, we have found that Cbt is able to bind to its own promoter region. In vivo analyses in Drosophila S2 cells indicate that Cbt probably acts as a negative regulator of its own expression during DC.

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Poster # 28

IDENTIFICATION OF *nmo*-RELATED GENES BY DIFFERENTIAL EXPRESSION ANALYSIS AND STUDY OF THEIR POTENTIAL ROLE IN THE *Drosophila* OMMATIDIAL ROTATION PROCESS

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One of the most important developmental events for the achievement of the final patterning of the *Drosophila* eye is the ommatidial rotation process. In this process, which occurs during late larval and early pupal stages, the developing ommatidia rotate 90^{o} in two 45^{o} steps. It is known that some signaling pathways like EGFR and Fz/PCP are involved in this process, however few genes have been proved to specifically affect it. One of these genes is *nemo* (*nmo*), a gene coding for a MAP-like protein kinase. *nmo* loss of function produces an arrest of ommatidia at 45^{o}, suggesting an essential role of this gene in the second rotation step. Whether there is a connection between *nmo* and the mentioned signaling pathways during ommatidial rotation is still unclear. In such a scenario and to gain further knowledge on the genes affected by *nmo* loss of function in this process, we performed differential expression analyses of *nmo* mutant third instar larval eye discs compared to wild type using expression microarrays. We have identified a total of 101 downregulated and 104 upregulated genes in *nmo* mutant eye discs ($p<0.05$) with respect to controls. Interestingly, many of these genes fell into functional categories which could be related to the ommatidial rotation process such as synthesis of extracellular matrix components, cytoskeleton biogenesis and organization, cell adhesion and signaling. Expression changes of four of these genes in *nmo* mutant eye discs have been validated by RT-qPCR. Furthermore, phenotypic analyses of loss and gain-of-function mutants of these genes indicate that they could have a role in the ommatidial rotation process. We will discuss these results in the context of *nmo* function and the signaling pathways involved in this specific developmental process.

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Poster # 29

NEW GENOMIC STRATEGIES TO ACCURATELY TRACK ACTIVELY TRANSCRIBING POLYMERASES IN *SACCHAROMYCES CEREVISIAE*

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Of special interest in the field of eukaryote functional genomics is the study of the precise behaviour of the enzymatic machinery responsible for transcription and how it is capable of modulating gene expression under different conditions. We have developed an *in vivo* endonuclease-based RNA Polymerase nascent transcription profiling strategy to improve the resolution of the genomic run-on technique (GRO), adapting it for high throughput transcriptome analysis, either analog (microarrays) or digital (RNA-seq). This provides us with valuable information about the location, density and orientation of actively transcribing-RNA Polymerases along the DNA, opening the possibility of a better characterization and modeling of the regulatory steps in RNA synthesis and RNA stability, genome-wide, with an unprecedented level of detail and precision in the budding yeast *Saccharomyces cerevisiae*.

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Poster # 30

DIFFERENTIAL EXPRESSION IN RNA-SEQ: A MATTER OF DEPTH

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Next generation sequencing technologies have made a breakthrough in the study and interpretation of cellular mechanisms. It is still a challenge to properly process the huge amount of data generated by these new technologies and interpret correctly the underlying information. Here, we give an overview of the possible biases of RNA-seq data and how these biases may affect differential expression. We also provide an empirical nonparametric method (NOISeq) to compute differential expression between two experimental conditions. This novel method is based on the joint distribution of log-ratio (M) and difference (D) values between counts and determines the probability for a gene (or exon or transcript) of being differentially expressed by comparing these M and D values for signal and noise. This algorithm can be used with or without replicates, unlike most of the existing methods, and does not rely on parametric assumptions about read counts distribution. NOISeq has been tested on public datasets and compared to several widely used methods for differential expression in RNA-seq data. We also discuss the influence of gene length or sequencing depth on differential expression.

Poster # 31

INTERPLAY BETWEEN GLIADINS AND *B. longum* CECT 7347 IN THE INTRACELLULAR CALCIUM HOMEOSTASIS IN INTESTINAL CELLS

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Celiac disease (CD) is an autoimmune enteropathy caused by gluten proteins (gliadin), which is associated with imbalances in the intestinal microbiota. There is limited knowledge of the interplay between gliadin peptides and beneficial intestinal bacteria in the alterations of intestinal epithelial architecture in CD. Previous of our studies showed that intestinal cultures challenged to digests of gliadins exhibited an up-regulation of the chemokine CXCR3 [G-protein coupled] receptor mRNA levels[1], and the gliadins-mediated inflammatory response was abolished by mobilization of intracellular calcium ($[Ca]_i$)[2]. The objective of this study was to evaluate the changes in the proteome of human intestinal epithelial cells (Caco-2) exposed to digests of gliadins, in the presence or absence of *B. longum* CECT 7347. There was detected an over-production of proteins, among other, that participate in the cellular signalling of gliadins (Regulator G-protein signalling 5, O15539); proteins associated with components of the cytoskeletal system, such as regulators of the actin filaments and cycle progression (RhoGEF and PH domain-containing protein 1, P98174). In cell cultures exposed to digests of gliadins inoculated with *B. longum* CECT 7347 there was quantified an over-production of proteins that participate in calcium homeostasis (EF-hand domain-containing protein, A8MZ26) controlling cytoplasmic Ca^{+2} levels into cells.
These findings suggest that *B. longum* CECT 7347 may reduce gliadin-induced toxicity to intestinal epithelial cells by regulating $[Ca]_i$. This effect could have important consequences in the pathogenesis of CD since the morphologic stability of intestinal epithelial tissue depends on the activation of T cells.

[1] Laparra and Sanz. J Cell Biochem 2010, 109, 801-807; [2] Laparra and Sanz. J Physiol Biochem 2010.

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Poster # 32

ANALYSIS OF *PARK16* IN FAMILIAL PARKINSON DISEASE

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Genome-wide association studies (GWA) have recently revealed the association of a new locus (*PARK16*) with the risk for Parkinson's disease (PD). The locus contains three complete genes: *RAB7L1*, *NUCKS1* and *SLC41A1*. The sequencing of these genes, and analysis of haplotypes in case-control studies have confirmed this association, and also had identified interesting mutations that could be related to PD highlighting the importance of those SNPs. In this work we analyzed by direct sequencing the coding regions of the three genes in a population of 65 patients with familial PD and 93 controls, identify new SNPs that may be involved in the onset of the disease, and determine the frequency of those described previously.

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Poster # 33

IN SILICO PHYLOGENETIC AND STRUCTURAL ANALYSIS OF PINK1

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Parkinson disease (PD) is the second most common neurodegenerative disorder and is characterized by the loss of dopaminergic neurons in the substantia nigra. Mutations in *PINK1* were shown to cause recessive familial PD and nowadays are proposed to be associated with the disease via mitochondrial dysfunction and oxidative damage. The *PINK1* gene comprises eight exons, which encode a ubiquitously expressed 581 amino acid protein that contains an N-terminal mitochondrial targeting domain and a serine/threonine protein kinase. This kinase domain is structurally similar to those of the Calcium-Calmodulin dependent kinases (CaCMK) and Dystrophia Myotonica Protein Kinase (DMPK). In this study, we investigate the origin of PINK1 and its phylogenetic relationships with CaMK and DMPK, the evolution of the protein among the phyla *cnidaria*, *nematoda*, *artropoda* and *chordata* and propose the better non-human model for PD studies. The study reveals the presence of PINK1 in other species from *cnidaria* to *vertebrates*, but no in plants or fungi, suggesting the late emergence in evolution, coincidental with the development of a complex nervous system. Besides, using structural predictions, we propose some explanations about the pathogenesis of some PD-causing mutations.

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Poster # 34

DIFFERENTIAL OVEREXPRESSION OF TRANSMEMBRANE SEGMENTS: A PROTEOMIC APPROACH

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About 25% of the open reading frames in most fully sequenced organisms have been estimated to encode □-helical membrane proteins (MP), which are responsible for the interchange of matter and information over the biological membrane, the lipid bilayer enveloping and defining the cell. Many are also prime contemporary or future drug targets, and it has been estimated that more than half of all drugs currently on the market are directed against □-helical membrane proteins. By contrast, it is still frustratingly hard to obtain high-resolutions three-dimensional structures, mainly due to the difficulties found in membrane protein overexpression.
MP overexpression is often toxic to the cell, thereby preventing biomass formation and severely reducing yields. Then, an understanding of the physiological response to overexpression is needed to improve such yields. Here, we analyzed the consequences of overexpressing two different transmembrane (TM) segments from single-spanning human MPs –glycophorin A (GpA) and surfactant protein C (SP-C)– fused to the C-terminus of staphylococcal nuclease in the bacterium *Escherichia coli* (BL21 strain) by using a proteomic approach. Proteomes with or without chimera proteins of total cell lysates were analyzed by one- and two-dimensional gel electrophoresis and mass spectrometry. Strikingly, the expression of the two chimera shows a protein yield significantly higher for the GpA-derived system compared to the SP-C one, and a preliminary differential expression pattern in other proteins that may participate in MP overexpression.

Poster # 35

gSNOW: A SET-ENRICHMENT BASED METHODOLOGY TO EXTRACT PROTEIN-PROTEIN INTERACTION NETWORKS ASSOCIATED TO SPECIFIC PHENOTYPES

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The cells can be considered as a system whose state and behavior is determined by an intricate network of interactions among all its elements. In order to study a cell state, it is necessary not only to focus on its individual components but also on its interactions and on the global organization of the network, i.e. its topology. Thus, methodologies capable of analyzing cell networks and translate its structure into functional information need to be developed and implemented. A common approach in the functional profiling of genome-scale experiments is to use a threshold-based approach that preselect a set of proteins/genes from a genome-scale experiment without taking into account the cooperation among cell components. However, systems biology inspired approaches focus on functional classes such as blocks of proteins/genes that act cooperatively (1). Within this framework, we present gradualSNOW (gSNOW), a new set enrichment-based network analysis method, inspired by the successful tool SNOW (2). gSNOW takes a list of ordered proteins/genes according to a phenotypic parameter, subdivides it in a series of additives partitions and, for each of these partitions, draws the minimum connected network (MCN), defined by theirs proteins/genes, based on protein-protein interactions (PPIs) network as scaffold. Finally, our method calculates several relevant topological network parameters and tests their statistical significance against a random distribution. PPI networks were obtained from the main public databases: IntAct, Biogrid and MINT. The aim of this approach is to find if there is any sublist that forms a MCN whose topology is far from being expected by chance.

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Poster # 36

DISSECTING SIGNALING PATHWAYS TO UNDERSTAND THE CONSEQUENCES OF GENE EXPRESSION CHANGES

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Understanding the aspects of the cell functionality that account for phenotypes is one of the main challenges in the analysis of the data produced in genomic experiments. Usually, curated functional modules, such as GO, KEGG, Biocarta, etc., have been used to study the collective activity of genes belonging to them as proxies of functional activity. However, approaches that consider the activity of the complete module have known limitations. In particular, in the case of pathways, such approaches do not exploit the knowledge of the relationships among genes.
Here we propose a new approach in which, instead of analyzing the activity of the pathway as a whole, we rather analyze the possible different functionalities resulting from the different ways in which signals can be transmitted across the pathway. The gene activity, estimated from the level of expression, can be used within a probabilistic context to calculate the probabilities of a signal to be transmitted from the input layer to the output layer in a pathway. Differential activity in distinct input/output connections will result in different functional activities in the pathway.
Several examples demonstrate the usefulness of the proposed approach.

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