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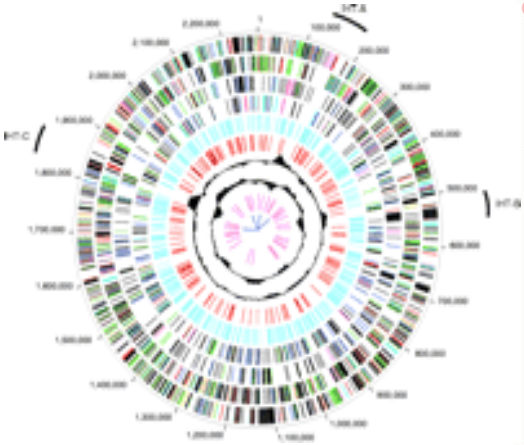

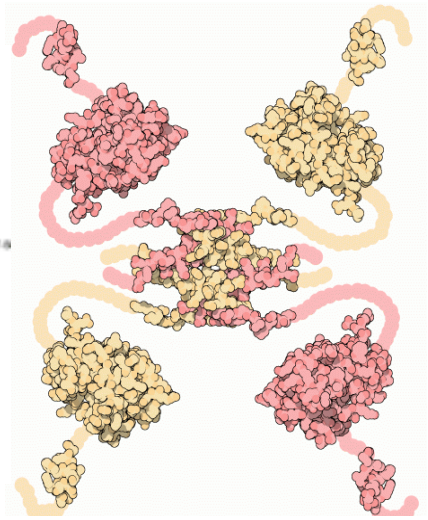

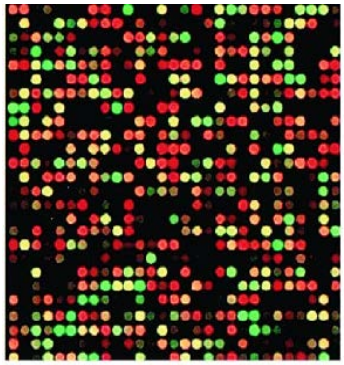

GENÓMICA Y PROTEÓMICA

DECIMA REUNIÓN DE LA RED VALENCIANA DE GENÓMICA Y PROTEÓMICA

10th Meeting of the Valencian Network for Genomics & Proteomics

Beyond the Human Genome

Salón de Actos Charles Darwin de las Facultades de Ciencias de la UV

7 de Noviembre de 2012

GENERALITAT VALENCIANA
Prometeo 2011/088
CONSELLERIA D'EDUCACIÓ

Patrocinadores:

MÁSTER UNIVERSITARIO EN BIOLOGÍA MOLECULAR, CELULAR Y GENÉTICA

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: Proteomics. Chairman: Manuel S. Pino.

9:30 h. Susana Cristobal. Dep. Clinical and Experimental Medicine, Linköping University, Sweden
Environmental proteomics: tools to address environmental questions

10:15 h. Juan Pablo Albar. ProteoRed-ISCIII, Coordinator. CNB/CSIC. Madrid
Human Proteome Project: Characterization of the proteins coded by the Chromosome-16 protein coding genes

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

11:30 h. Session II: Genomes. Chairwoman: Rosario Gil.

11:30 h. Antonio Granell. IBMCP/CSIC. Valencia.
The tomato genome and beyond

12:15 h. Alex Mira. CSISP- GV. Valencia
Studying the Human Microbiome through Metagenomics and Next-Generation Sequencing

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

15:00 h. Session III: Epigenomics. Chairman: Jordi Pérez Tur.

15:00 h. Sarah Djebali. Centre de Regulació Genòmica (CRG). Barcelona
The transcriptional landscape of the human genome. ENCODE project.

15:45 h. Marc A. Marti-Renom. Structural Genomics Group. Centre Nacional D'Anàlisi Genòmic (CNAG) & Centre de Regulació Genòmica (CRG). Barcelona.
Structure determination of genomes and genomic domains by satisfaction of spatial restraints

16:30 h. Special session: Emerging technologies. Chairman: Ismael Mingarro.

16:30 h. Raquel Cuellar. Field Application Specialist de Biorad Laboratories SA.
Droplet Digital PCR: Molecular Biology in High Resolution.

17:00 h. Antonio Serna Sanz. *ABSCIEX*
Enabling the next generation of Mass Spectrometry-based Quantitation Workflows

17:30 h. Poster session. Coffee and cakes available.

18:30 h. End of the meeting.

Plenarias
Plenary sessions

Plenary Session I

ENVIRONMENTAL PROTEOMICS, TOOLS TO ADDRESS ENVIRONMENTAL QUESTIONS

Susana Cristobal

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Environmental proteomics research is focused on the analysis of an organism's proteome and the detection of changes in the level of individual proteins in response to environmental stressors. Pollutants are among other stressors that provoke global changes in cellular protein expression. Proteomics-based methods have been applied at our lab for the screening of protein expression signatures of exposure to model pollutants and in both, laboratory and field experiments. We have found complex and robust biomarkers for marine pollution assessment. This technology has been applied in several European projects such as BEEP, which was a research programme studying the biological effects of environmental pollution in marine coastal; or Prestepse, which performed an integrated evaluation of the effects after the *Prestige*'s oil spill, which was one of the largest oil spill in the marine history. In our work, peroxisomal proteomics has been applied to obtain protein expression signatures of exposure to individual pollutants and to anthropogenic pollution in field experiments. These identifications have opened opportunities to study the poor understood pathways affected by xenobiotics such as flame retardants, bisphenol, and phthalate. We have also applying a peptide profiling by magnetic particle-assisted chromatography and MALDI-TOF mass spectroscopy. Using the haemolimph from invertebrates or plasma from fish that contains a complex array of proteolytically derived peptides, relevant information can be provided to correlate with biological events occurring in the entire organism. Finally, the commercial applications of nanotechnology expand more rapidly than the scientific knowledge on NP exposure. Thus, the use of nanomaterials may pose unknown risks to human and environmental health and our exposure to nanoparticles has started to consider novel environmental risks. Our research is now focused on analyzing the effects of exposure to nanoparticles combining proteomics and lipidomics. This strategy could provide a global understanding of the nanoparticles interactions with biomolecules, cellular functions impaired, and modification in cell membrane fluidity by an integrated analytical approach.

Plenary Session I

HUMAN PROTEOME PROJECT: CHARACTERIZATION OF THE PROTEINS ENCODED BY THE CHROMOSOME-16 PROTEIN CODING GENES

SI. Gharbi[1,] V. Segura[2], JA. Medina-Aunon[1], E. Guruceaga[2], C. González-Tejedo[1], MM Sánchez del Pino[3], F. Canals[4], M. Fuentes[5], I. Casal[6], S. Martínez-Bartolomé[1], F. Elortza[7], JM Mato[7], JM Arizmendi[8], J. Abian[9], E Oliveira[10], C. Gil[11], F. Vivanco[12], F. Blanco[13], FJ Corrales[2] and **JP Albar[1*].***

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The Chromosome 16 Consortium is integrated in the global initiative *Human Proteome Project* that aims to develop an entire map of the proteins encoded following a gene-centric strategy (C-HPP) to make progress in the understanding of human biology in health and disease (BD-CHPP). The structure of the consortium involves 15 groups organized in five working sections, namely Protein Microarray and Peptide Standards, Selected Reaction Monitoring team, Protein Sequencing team, Bioinformation Support team and Clinical healthcare and biobanking teams.

A description of chromosome 16 based on knowledge repositories has been analysed; the chromosome contains 862protein coding genes including 751 "known" and 111 "unknown" gene products (log(e)>-5 GPMDB), participating in 116 OMIM diseases. Lymphocytes B, epithelial cells and fibroblasts were selected for further studies as transcriptomic evidences suggest that most chromosome 16 protein coding genes are expressed in these cell lines. For analytical purposes, the 862 proteins have been clustered in two groups according to the robustness of the evidences supporting their MS observation, It is expected to set SRM assays for the top rank third of chromosome 16 proteins in both protein groups.

The process is being developed on a multicentric configuration, assuming the standards and integration procedures already available in ProteoRed-ISCIII, which are encompassed with HUPO initiatives. A biobanking initiative has been launched in collaboration with the Spanish National Biobanking Network to optimize methods for sample collection, management and storage under normalized conditions and to define QC standards.

As a proteomic pilot study, we are defining a comprehensive proteomic map based on high-resolution data dependent mass spectrometry to define the protein coverage of the Chr16. This study will permit to assist the development of MRM methods for the quantitative targeted strategy. We are dissecting the human T lymphoblast cell line (Jurkat T cells) using in a first instance a MUDPIT type approach, with in-solution digestion and off-line pre-fractionation by RP-HPLC-Basic. Then, to gain better insight into integral membrane proteins, our analysis is combined with strong detergent protein extraction and 1D-gel-digestion. In all our workflows, data is acquired using a high resolution and accurate mass 5600 TripleTOF LC/MS/MS system (AB SCIEX) on long HPLC runs (4 hrs). This global proteome is then fed into our in-house MIAPE-Extractor tool that compiles all the Mascot-searched data and extracts specific MIAPE information relative to Chr16. This logistic permits the integration of the versatile formats and workflows that can all converge into providing a fast and complete understanding of the proteome of the cell model of interest. We anticipate that this rapid screen will greatly complement our efforts in mapping the Chr16.

Plenary Session II

THE TOMATO GENOME AND BEYOND

Antonio Granell

Instituto de Biologia Molecular y Celular de Plantas de Valencia, C.S.I.C (PUV. Ciudad Politécnica de la Innovación. Escalera 8E. C/ Ingeniero Fausto Elio s/n, 46022 Valencia. Spain.

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Tomato (*Solanum lycopersicum*) is a major crop plant and a model system for fruit development. A high-quality genome sequence of domesticated tomat and a draft sequence of its closest wild relative, *Solanum pimpinellifolium* have been published recently. This hallmark opens the way to understanding evolution and adaptation of tomato and other *Solanaceae* members to a wide range of conditions and also to identify genes underlying important traits for tomato and other related crops. I will present the sequence of tomato and the wild relative and give some examples of how the sequence is already been used to identify relevant genes that affect fruit quality. This is particularly important since the Solanum lineage has experienced two consecutive genome triplications: one that is ancient and shared with rosids, and a more recent one. These triplications set the stage for the neofunctionalization of genes controlling fruit characteristics, such as colour, fleshiness, etc.

I will present the case of the identification of the tomato Uniform ripening (U) locus, a Golden 2-like (GLK) transcription factor with a loss of function mutation in most cultivars used for consumption and how we found out that this gene is responsible for the pattern and intensity of green fruit color. Enhancement of fruit photosynthesis mediated by increased levels of active GLK in the fruit resulted in green fruits with more starch and red fruits with more sugars and chromoplasts accumulating lycopene. Our research revealed that sometimes breeding for one trait has important tradeoffs for others but also open new approaches to increase fruit quality by enhancing fruit photosynthesis through GLK genes.

Plenary Session II

STUDYING THE HUMAN MICROBIOME THROUGH METAGENOMICS AND SECOND-GENERATION SEQUENCING

Alex Mira, *Pedro Belda, Alfonso Benítez, Raúl Cabrera, Áurea Simón*

Área de Genómica y Salud
Centro Superior de Investigación en Salud Pública, Valencia
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Bacteria inhabiting the human body play important roles for health that are still not fully understood. Those bacteria inhabit our gut, respiratory tract, skin, oral cavity, urinary tract, etc, and can be considered as an organ whose function needs to be elucidated. An important fraction of the human-associated microbiota has not been cultured and therefore the use of DNA-based techniques has revolutionized the research in the field. Metagenomics is the study of all genes present in the bacterial community obviating the need for culture and has traditionally performed by cloning of microbial DNA in bacterial hosts able to grow in the laboratory. The combination of metagenomic techniques with second-generation sequencing techniques like pyrosequencing or Illumina sequencing has allowed researchers to describe the composition and genetic repertoire of the human microbiome to an unprecedented level of detail. We have applied these techniques to describe the microbiota associated to the respiratory tract, the human breast milk, the stomach and specially the oral cavity. Comparing the oral bacterial diversity in individuals that have never suffered from dental caries we have identified some commensal bacteria that appear to protect the teeth against caries-associated species. In addition, we have described the bacteria present in cavities at different stages of the disease, the specialization of bacteria on different microniches of the mouth and the full functional profile of bacteria inhabiting the tooth surface. Recently, the massive sequencing of RNA from dental plaque samples have allowed us to study the active bacteria and genes during the development of the oral biofilm. We have applied this metatranscriptomics approach to detect the active species after a meal, in an attempt to narrow down the list of species responsible for acid production and dental caries. Finally, the combination of flow-citometry and cell sorting with massive sequencing has served us to identify the bacteria recognized by different antibodies in saliva. Thus, the study of the human microbiome is moving from a descriptive period to more functional approaches where the expression of individual genes and the interaction with the immune system will serve to unravel the function of our microbial partners in health and disease.

Funding for the projects described in this work was provided by grants Microgen CSD2009-00006 from the Consolider-Ingenio program and SAF2009-13032-C02-02 from the Spanish Ministry of Science and Innovation.

Plenary Session III

THE TRANSCRIPTIONAL LANDSCAPE OF THE HUMAN GENOME

Sarah Djebali[1]*, Carrie A. Davis[2]*, Angelika Merkel[1], Alex Dobin[2], Timo Lassmann[7], Ali M. Mortazavi[5,8], Andrea Tanzer[1], Julien Lagarde[1], Wei Lin[2], Felix Schlesinger[2], Chenghai Xue[2], Georgi K. Marinov[5], Jainab Khatun[4], Brian A. Williams[5], Chris Zaleski[2], Joel Rozowsky[13,14], Maik Röder[1], Felix Kokocinski[12], Rehab F. Abdelhamid[7], Tyler Alioto[1], Igor Antoshechkin[5], Michael T. Baer[2], Nadav S. Bar[17], Philippe Batut[2], Kimberly Bell[2], Ian Bell[3], Sudipto Chakrabortty[2], Xian Chen[11], Jacqueline Chrast[10], Joao Curado[1], Thomas Derrien[1], Jorg Drenkow[2], Erica Dumais[3], Jacqueline Dumais[3], Radha Duttagupta[3], Emilie Falconnet[9], Meagan Fastuca[2], Kata Fejes-Toth[2], Pedro Ferreira[1], Sylvain Foissac[3], Melissa J. Fullwood[6], Hui Gao[3], David Gonzalez[1], Assaf Gordon[2], Harsha Gunawardena[11], Cedric Howald[10], Sonali Jha[2], Rory Johnson[1], Philipp Kapranov[3,16], Brandon King[5], Colin Kingswood[1], Oscar J. Luo[6], Eddie Park[8], Kimberly Persaud[2],Jonathan B. Preall[2], Paolo Ribeca[1], Brian Risk[4], Daniel Robyr[9], Michael Sammeth[1], Lorian Schaffer[5], Lei-Hoon See[2], Atif Shahab[6], Jorgen Skancke[1,17], Ana Maria Suzuki[7], Hazuki Takahashi[7], Hagen Tilgner[1], Diane Trout[5], Nathalie Walters[10], Huaien Wang[2], John Wrobel[4], Yanbao Yu[11], Xiaoan Ruan[6], Yoshihide Hayashizaki[7], Jennifer Harrow[12], Mark Gerstein[13,14,15], Tim Hubbard[12], Alexandre Reymond[10], Stylianos E. Antonarakis[9], Gregory Hannon[2], Morgan C. Giddings[4,11], Yijun Ruan[6], Barbara Wold[5], Piero Carninci[7], Roderic Guigó[1], Thomas R. Gingeras[2,3]

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Eukaryotic cells make many types of primary and processed RNAs that are found either in specific sub-cellular compartments or throughout the cells. A complete catalogue of these RNAs is not yet available and their characteristic sub-cellular localizations are also poorly understood. Since RNA represents the direct output of the genetic information encoded by genomes and a significant proportion of a cell's regulatory capabilities are focused on its synthesis, processing, transport, modifications and translation, the generation of such a catalogue is crucial for understanding genome function. Here we report evidence that three quarters of the human genome is capable of being transcribed, as well as observations about the range and levels of expression, localization, processing fates, regulatory regions and modifications of almost all currently annotated and thousands of previously unannotated RNAs. These observations taken together prompt to a redefinition of the concept of a gene.

This work was supported by the National Human Genome Research Institute (NHGRI) production grants number U54HG004557, U54HG004555, U54HG004576 and U54HG004558, and by the NHGRI pilot grant number R01HG003700. It was also supported by the NHGRI ARRA stimulus grant 1RC2HG005591, the National Science Foundation (SNF) grant number 127375, the European Research Council (ERC) grant number 249968, a research grant for the RIKEN Omics Science Center from the Japanese Ministry of Education, Culture, Sports, Science and technology, and grants BIO2011-26205, CSD2007-00050, and INB GNV-1 from the Spanish Ministry of Science.

STRUCTURE DETERMINATION OF GENOMES AND GENOMIC DOMAINS BY SATISFACTION OF SPATIAL RESTRAINTS

Marc A. Marti-Renom

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The genome three-dimensional (3D) organization plays important, yet poorly understood roles in gene regulation. Chromosomes assume multiple distinct conformations in relation to the expression status of resident genes and undergo dramatic alterations in higher order structure through the cell cycle. Despite advances in microscopy, a general technique to determine the 3D conformation of chromatin has been lacking. We developed a new method for the determination of the 3D conformation of chromatin domains in the interphase nucleus, which combines 5C experiments with the computational Integrative Modeling Platform (IMP). The general approach of our method, which has been applied to study the 3D conformation of the a-globin domain in the human genome [1] and the *Caulobacter crescentus* whole genome [2], opens the field for comprehensive studies of the 3D conformation of chromosomal domains and contributes to a more complete characterization of genome regulation.

[1] D. Baù et al. Nat Struct Mol Biol (2011) 18:107.
[1] M.A. Umbarger, et al. Molecular Cell (2011) 44:252

Special session

DROPLET DIGITAL PCR: MOLECULAR BIOLOGY IN HIGH RESOLUTION

Raquel Cuellar. Field Application Specialist de Biorad Laboratories SA.

Droplet digital™ PCR is the third generation of PCR technology. In digital PCR, the target molecules in a DNA sample are isolated in separate chambers by dilution, partition, or a combination of both. Those separate chambers can be the wells of a microtiter plate, capillaries, micro- or nanoarrays, or as in the case of Bio-Rad droplet digital PCR system, 20000 nanodroplets. The goal is to have either one or no molecules in each droplet, so that when we perform PCR we have a simple positive or negative amplification result. The initial amount of target molecules is then directly quantified by counting the number of droplets with a positive amplification.

Droplet digital PCR provides researchers with a new tool for the detection of rare mutations, including distinguishing rare sequences in tumors, precise measurement of copy number variation, and absolute quantification of sequencing libraries, especially trace libraries from precious samples.

DROPLET DIGITALTM PCR: BIOLOGÍA MOLECULAR DE ALTA RESOLUCIÓN.

La Droplet digital™ PCR is la tercera generación de la tecnología PCR. Con esta técnica, la muestra de ADN se aísla en 20000 nanogotas. El objetivo de esta partición, es tener una o ninguna molécula de ADN en cada gota, de modo que cuando cada gota es amplificada por PCR se obtiene simplemente un resultado positivo o negativo. La cantidad inicial de moléculas se cuantifica directamente contando el número de gotas con resultado positivo en la amplificación del ADN.

La Droplet Digital PCR ofrece a los investigadores una nueva herramienta, más precisa y sensible que la Real Time PCR y la PCR tradicional, para la detección y cuantificación de bajos niveles de patógenos, detección de mutaciones raras, la medición de la variación del número de copias, el estudio de la expresión génica en células únicas, y la cuantificación absoluta de bibliotecas genómicas.

Info en youtube:

http://www.youtube.com/watch?v=GB4wcQsCawU&feature=related

http://www.youtube.com/watch?v=wBlV-MBvftc&feature=related

Special session

ENABLING THE NEXT GENERATION OF MASS SPECTROMETRY-BASED QUANTITATION WORKFLOWS

Antonio Serna Sanz

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In the last years Proteomics-based Mass Spectrometry has turned from untargeted to targeted analysis. Quantitation data have become more relevant to nowadays research as clearly reflected in the amount of projects that demand these kinds of workflows. Likewise technology did evolve to increase throughput in the analysis of complex proteomes, so must do to enable the quantitation of large numbers of protein sets in the best possible fashion. Here we introduce a new workflow for Mass Spectrometry that will enable this step forward. This workflow is based in a Data Independent Acquisition with discrete transmission windows across broad mass ranges. The type of information generated is facilitating the rapid generation of methods for quantitating numbers of proteins so far unreachable through classical targeted approaches.

Posters

Poster # 1

INTRACELLULAR PROTEOLYSIS AND NEOPLASIA: UBIQUITIN-PROTEASOME SYSTEM, CELL CYCLE AND CANCER

***Francisco Torrens*[1] *and Gloria Castellano*[2]**

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Affinity may include the ease of traverse *via* chreodes to the effector. Lag may be because of the time or concentration needed for a drug to displace transmitter molecules from the chreodes. Molecules unfit for the system may be excluded from the effector by not fitting the chreode patterns. Persistence may be because of the lingering of molecule in the chreodes after washout. Enzyme catalytic products leave the active site at a faster rate *via* chreodes, minimizing the retarding effect of diffusion-controlled rate limitation. Protein kinase-C-dependent ubiquitylation promotes non-proteolytic inactivation of suppressor Fbw7α which, although expressed in mature *Xenopus* eggs arrested in metaphase II, is nonfunctional explaining why cyclin-E can be stockpiled in mitotic-like phase. Provisional conclusions follow. (1) The DDX11 shares sequence with FANCJ and DEAH-box helicases XPD/RTEL, which contain one Fe/S cluster between domains IA/II; FANCJ/XPD connect to genetic-instability syndromes Fanconi anaemia and xeroderma pigmentosum, and inactivation increases cancer risk. Inactivation of DDX11 causes genetic instability combining Fanconi anaemia/Roberts syndrome; given DDX11-deficient cell hypersensitivity for mitomycin-C/campothecin, which interfere with deoxyribonucleic acid replication, DDX11 functions at replication-coupled acid repair/sister chromatid cohesion interface. (2) In *Xenopus* eggs/embryos, Fbw7α inactivation correlates with protein kinase-C-dependent polyubiquitylation, which is maintained until end of early rapid cleavage cycles where cyclin-E undergoes degraded. Kinase-C-dependent negative regulation of Fbw7α is conserved during human somatic cell cycles, resulting in cyclin periodic expression. In mechanism for Fbw7α regulation, functions change by kinase deregulation, which occurs in tumour types. (3) In mechanism controlling B-cell lymphoma-6 protein stability, FBXO11 mutations/deletions in human diffuse large B-cell lymphomas contribute to lymphomagenesis, *via* 6-protein stabilization. (4) The Fbxo9-telomere maintenance-2/interacting protein-1 axis is phosphatidylinositol-3 kinase-related kinase regulatory hub, whose deregulation contributes to multiple-myeloma development/progression. (5) Cancer jun-B implication is context dependent: besides cell proliferation inhibition/senescence, it suppresses tumour; its murine overexpression inhibits B-cell transformation by oncogene v-abl. It contributes to tumour phenotype; as disrupted passage *via* mitosis leads to chromosome missegregation and aneuploid progeny production, it favours that jun-B overexpression in late G_2 represent oncogenic mechanism. (6) Pharmacological/genetic inactivation of GSK3-FBXW7-jun-B axis induced jun-B accumulation, in G_2/M, and entailed *DDX11* transcriptional repression leading to premature sister chromatid separation. Abnormal phenotype, because of GSK3β/jun-B/DDX11 pathway deregulation, is phenocopied in anaplastic lymphoma kinase-positive anaplastic large-cell lymphomas. Mitosis progression and chromatid cohesion are regulated *via* GSK3/SCF^{FBXW7}-mediated jun-B proteolysis, essential in maintaining genetic fidelity during mitosis. (7) Zn^{2+} negatively affects growth, cytotoxicity and expression of *T. vaginalis* proteinases related to prostatic cell interaction. Further work will deal with vitamin-D relevance and stem-cell connection with cancer.

Funding for the projects described in this paper was provided by grant BFU2010-19118 from the Spanish Ministerio de Ciencia e Innovación.

Poster # 2

Planococcus citri NESTED ENDOSYMBIOSIS: GOING DEEPLY INTO FUNCTIONAL AND EVOLUTIONARY ASPECTS.

***Sergio López-Madrigal*[1]*, Amparo Latorre*[1,2]*, Manuel Porcar*[3]*, Andrés Moya*[1,2]*, Rosario Gil*[1]

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Many insect species maintain obligatory mutualistic symbioses with intracellular bacteria that complement their unbalanced diets. In some cases, two bacteria coexist in the same host, establishing a consortium that is needed for the system fitness. Coexisting bacteria can be occupying different cells or even the same cell cytoplasm. In this context, mealybugs of the subfamily Pseudococcinae present a complex nested endosymbiotic system where a β-proteobacterium, *Tremblaya princeps,* harbours a γ-proteobacterium, *Moranella endobia*. Recent genome sequencing of two strains (PCIT and PCVAL) of *T. princeps* and *M. endobia* from *Planococcus citri* (Risso) revealed an unprecedented functional complementation between them. Showing the smallest bacterial genome described so far (139kb), *T princeps* has lost the ability for DNA replication and transcription, as well as part of its translational machinery and most metabolic functions (although it is still able to synthesize several essential amino acids). In contrast, *M. endobia* still retains most of the essential functions lost by *T. princeps*. Thus, both bacteria could not be considered as independent organisms but as part of an unprecedented composed entity. Genome-driven reconstruction of the consortium functions, as well as comparative genomics and evolutionary analyses on these consortium genomes are revealing some clues of the meaning and evolutionary path of this peculiar system.

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Poster # 3

EFFECT OF ANTIBIOTIC THERAPY ON THE DIVERSITY AND FUNCTIONAL ACTIVITY OF THE HUMAN INTESTINAL MICROBIOTA.

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The human intestinal microbiota is involved in a large number of essential functions for the host such as food processing, regulating the growth of intestinal epithelium, development of immune system, synthesis of essential vitamins or protection against pathogens. Antibiotic use has been crucial to treat bacterial infections for over half a century. The problem is that antibiotic therapies not only affect the pathogen but also to normal bacteria of the microbiota, which can trigger some health problems such as antibiotic-associated diarrhea (AAD), since the suppression of the beneficial members can allow colonization and overgrowing of some pathogens as *Clostridium difficile*. Also, antibiotic treatments promote the expansion of antibiotic-resistant strains, turning the gut ecosystem in a reservoir of resistance genes, what is currently a serious problem in public health.

Most of the recent studies to describe the effect of antibiotics in the gut microbiota have focused on the diversity changes based on analysis of 16SrDNA gene. We analyzed the effect of antibiotics in the human gut microbiota from a more complete perspective by means of integration of total microbiota (16SrDNA), active microbiota (16SrRNA), metagenome, and metatranscriptome information obtained from a follow-up of a beta-lactam antibiotic course.

We have found significant changes in the composition of both active and total bacteria, with a great reduction in diversity, being the dominant phyla during treatment Bacteroidetes and Proteobacteria. Also, we found a partial recovery of the initial microbiota after treatment but some members still remained lost. The metatranscriptomic results showed significant changes in the expression of genes involved in responding to treatment such as those related with the activation of transport systems or those related with the synthesis of compounds to maintain the integrity of the cell wall, which have been described as mechanisms involved in β-lactam resistance.

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Poster # 4

THE ROLE OF THE CYTOPLASMIC EXONUCLEASE XRN1 IN SACCHAROMYCES CEREVISIAE TRANSCRIPTION

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In eukaryotes, mRNA is transcribed in the nucleus, exported to the cytoplasm, translated and eventually degraded. Thus, gene regulation has been historically described as a linear process. To maintain the appropriate level of mRNA in the cytoplasm, it is necessary to coordinate the rates of transcription and mRNA degradation. It has been shown that Rpb4-Rpb7 complex is involved in transcription, export, translation and mRNA degradation, probably acting in the regulation of gene expression as a global coordinator [(1)]. Thus, some kind of transcriptional information is transferred from the nucleus to the cytoplasm. For a proper homeostatic control, there must be an information flow related to the stability of mRNAs from the cytoplasm to the nucleus as well. A candidate for this reporter role is the exonuclease Xrn1, which has exonuclease activity 5' to 3', and is responsible for the major mRNA degradation pathway in yeast. *XRN1* disruption is not lethal, but provokes multiple effects. In this work, using the techniques of *Genomic Run On* [(2)] and *Transcriptional Shut-off* [(3)], we found that in *xrn1* mutants, transcription rates are reduced with a compensatory increase in the mRNA half-lives, suggesting that transcription and degradation are coupled and communicated processes.

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Poster # 5

TRANSCRIPTOME OF *PENICILLIUM DIGITATUM* DURING THE INFECTION OF ORANGES

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Penicillium digitatum is the causal agent of green mould rot of citrus fruit, and represents the major postharvest pathogen of citrus fruit in Mediterranean regions. In this study, with the aim of better understanding the infection process on oranges, we used massive parallel pyrosequencing with 454 Titanium technology to perform a global RNA-Seq transcriptomic analysis of *P. digitatum* in time series from 0 to 48 h after pathogen inoculation, where first symptoms of disease appeared. To identify the putative origin of the reads, two reference genomes were used: (i) the *Citrus sinensis* Genome Assembly (JGI v1.0; these sequence data were produced by the US Department of Energy Joint Genome Institute http://www.jgi.doe.gov/ in collaboration with the user community), and (ii) the first draft of the *P. digitatum* isolate Pd1 genome with a 20X genome coverage, elaborated in house. All sequence reads from a total of four libraries were assembled in a reference transcriptome containing 24410 contigs or putative genes. About 30% of those putative genes were assigned to *P. digitatum* and about 70% to *C. sinensis*. The number of *P. digitatum* putative genes increased as the infection progressed, whereas citrus genes showed the opposite trend. Quantitative reverse transcription PCR profiling of selected fungal genes revealed dynamic expression patterns during infection of orange fruits.

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Poster # 6

HIGH RESOLUTION GENOMIC RUN-ON ANALYSIS OF YEAST TRANSCRIPTION

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Of special interest in the field of eukaryote functional genomics is the study of the behavior of the enzymatic machinery responsible for transcription and how is it capable of modulating gene expression under different conditions. We have implemented the well-established genomics methodology genomic run-on (GRO)[1] for its use with high-density tiling microarrays in the yeast *Saccharomyces cerevisiae*. This new approach is based in the use of biotin-UTP during run-on elongation (BioGRO) after RNase depletion of pre-existing RNA. The method tracks every nascent transcript readily as it is been generated by RNA Polymerases (RNAPs), providing us with new insights into the precise positioning of actively transcribing RNAPs along transcript regions. Overall, the constraints that nucleosomes pose for RNAP passage becomes clear by the appearance of transcription peaks just after each of the first four nucleosomes. Positioning of active RNAPs and nucleosomes show alternative peaks along the body of transcripts *in vivo*, thus confirming previous observations of nucleosome-mediated backtracking and reactivation[2]. In-depth analysis reveals that this pattern varies greatly with the level of transcription and between gene functional categories, and that it becomes altered in *rpb4* or *dst1* mutants. Conversely, we also checked if changing the chromatin context had a direct effect in the density of RNAPs. We chose to disrupt *ISW2*, a member of the ISWI family of ATP-dependent chromatin remodelers, previously reported to have a nucleosome packing activity consistent of sliding nucleosomes towards the 5' nucleosome free regions[3]. Our results show a shift of the active polymerase density peaks towards the 3' of genes, consistent with the loss of *ISW2* packing activity. Active RNAP density profiling at the end of genes show that RNAP transcribes further downstream of the annotated polyadenilation sites, specially in shorter genes, and reveals previously unknown features that involve pausing and reactivation dynamics influenced by terminal nucleosomes surrounding the cleavage and polyadenilation site. Our study focuses also into RNA Polymerase III nascent transcriptome, and here we present the first dataset of nascent transcription rates for this RNAP, as well as a detailed analysis of its precise contribution, genome-wide, by means of selective inhibition experiments that shed light into the interplay between RNAPs II and III.

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Poster # 7

REQUIREMENT OF HISTONE H2B DEUBIQUITINATION IN SUS1 FUNCTIONS COORDINATING TRANSCRIPTION AND EXPORT.

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Efficient coupling between mRNA synthesis and export is essential for gene expression. Sus1, a component of the SAGA and TREX-2 complexes, is involved in both transcription and mRNA export [1]. Moreover, Sus1 is part of the SAGA DUB module involved in histone H2B deubiquitination. Several DUB components are necessary for the export of SAGA-regulated genes. However, the role of the DUB in mRNA export and gene tethering to the Nuclear Pore Complex is unclear. To gain knowledge on the role of ubiquitination in SAGA-TREX-2 functions, we monitor the effect of H2B deubiquitination in transcription coupled to export in order to determine the specific histone H2B deubiquitination role in Sus1 physical associations and Sus1 cellular functions. To do that we use the H2BK123R mutant strain, which is not ubiquitinable. We will also analyse the contribution of H2B deubiquitination to mRNA export. Among the SAGA factors that contribute to global H2B ubiquitination levels, Spt8 has a prominent role. Spt8 is a distinctive subunit of SAGA, being absent in the SLIK complex. SLIK is a SAGA-type histone acetyltransferase complex that contains a smaller truncated form of Spt7 and lacks Spt8. Cells lacking Spt8 have increased level of ub-H2B [2]. We analyse the specific role of Spt8 in Sus1 association with SAGA/TREX-2 and the activities of these complexes in this mutant and the impact of the absence of *SPT8* in chromatin recruitment to the NPC.

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Poster # 8

TREX-2 mRNA export complex and SAGA dependent deubiquitylation

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Evidence indicates that transcription and mRNA export are linked processes. The molecular mechanisms of this coordination are not clear however. Sus1 physically couples activated genes with the Nuclear Pore Complex because it is part of two protein complexes: SAGA, a transcriptional co-activator and TREX-2 that functions in mRNA biogenesis and export. The coordinated action of SAGA and TREX-2 is required for gene expression. Here we show that Sem1 influences Sus1 role in mRNA export and TREX-2 stability. Interestingly, wide analyses of gene expression reveal that Sem1 and Sus1 have partial overlapping functions also in transcription. In the absence of Sem1, expression of some SAGA-dependent genes is compromised with a concomitant decrease of RNA polymerase II recruitment to promoters. Interestingly, ChIP experiments revealed a distinct dependency for SAGA subunits recruitment on Sem1. While absence of Sem1 lowers Ada2 and Taf9 recruitment to *GAL1* promoter, DUB module association remains intact. However its in vitro enzymatic activity is dramatically decreased. Moreover, absence of TREX-2 component Thp1 also leads to a reduction in SAGA-dependent DUB activity. These results unveil a new role for Sem1 in influencing activation of SAGA-dependent H2B deubiquitylation likely mediated by stabilization of TREX-2 complex and SAGA whole assembly.

Poster # 9

APPLICATION OF MASS SPECTROMETRY TECHNIQUES FOR ESCHERICHIA COLI HEAT-LABILE TOXIN-SUBUNIT B CHARACTERIZATION

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Toxigenic *E. coli* strains cause infections in human and domestic animals and have been classified into different categories, including enterotoxigenic *E. coli* (ETEC), which is the most important pathogen of diarrhea in infants, children, and adults. The possibilities of characterizing the heat-labile enterotoxin (LT) of enterotoxigenic *Escherichia coli* (ETEC) by liquid chromatography electrospray mass spectrometry (LC/ESI-MS) and matrix-assisted laser desorption with time-of-flight mass spectrometry (MALDI-TOF-MS) were investigated.

The B sub-unit from recombinant *E. coli* (expression in *Pichia pastoris*) can be detected by LC/ESI-MS and the charge envelope signals can be observed; LC/ESI-MS and MALDI-TOF-MS analysis allowed the acquisition of labile toxin subunit B (LTB) molecular weight and preliminary structural characterization of LTB toxin. MALDI-TOF analysis after reduction and alkylation of the protein evidenced the presence of one disulfide bond in the structure of the protein. Confirmatory analysis was carried out by detection of most of the tryptic fragments of the B subunit by MALDI-TOF-MS, obtaining total coverage of the protein sequence. Possible biovariations in the toxin can mostly be determined by sequencing, where an increase of molecular mass in the N-terminal side of the protein was identified. This modification may be due to an O-GlcNAc-1-phosphorylation.

Poster # 10

ITRAQ AND DIGE IN THE ECOPHYSIOLOGICAL CHARACTERIZATION OF ZEBRA MUSSEL POPULATIONS (DREISSENA POLYMORPHA) INVADING THE EBRO AND THE JÚCAR HYDROGRAPHIC BASINS

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The invasion of zebra mussel (*Dreissena polymorpha*) in the ecosystems involves a serious environmental risk, at both ecological and socioeconomic levels. The control of the zebra mussel invasion requires previous knowledge about the main colonization strategies and dispersal ability, as well as a comprehensive study of the mechanisms involved in coping with environmental factors. The study included six populations sampled at diverse locations from the Ebro and Júcar basins, differing in their water quality. We have used osmoregulatory and ionoregulatory characters, defences against metal toxicity, and a general health status index for the ecophysiological characterization of zebra mussel populations. Furthermore, proteomics (DIGE and iTRAQ methodology) allowed us to find some of the proteins involved in the physiological adaptation of *Dreissena polymorpha* to the environmental conditions of the studied sites.

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Poster # 11

FRANKENSTEIN RTS-OCELLATUSIN. EVIDENCE FOR THE INDEPENDENT ORIGIN OF RGD AND KTS/RTS DISINTEGRINS

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Snake venoms contain a complex variety of pharmacologically active compounds. Disintegrins are a family of small (41-83 amino acids), cysteine-rich polypeptides broadly distributed in the venoms of vipers and rattlesnakes. The disintegrin family comprises potent and specific antagonists of β_1 and β_3 integrin receptors. Their inhibitory activity depends on the appropriate pairing of cysteines, which determines the conformation of an inhibitory loop that harbors an active tripeptide located on a mobile loop. The sequence of the active tripeptide primarily determines the specificity towards integrin receptors. Hence, disintegrins bearing the XXD motif at the tip of an 11-residue mobile loop distinctly block β_1 and β_3 integrins. KTS- and RTS-distintegrins represent selective $\alpha_1\beta_1$ inhibitors. The $\alpha_1\beta_1$ integrin is involved in VEGF-triggered tumor angiogenesis; its selective blockade is thus a desirable goal for killing certain kinds of cancer cells by starving them to death. NMR studies of $\alpha_1\beta_1$-blocking KTS and RTS disintegrins have revealed that their integrin binding loops and C-terminal tails form conformational functional epitopes that display concerted motions. The shape and size of the 9-residue integrin-binding loop, along with its composition, flexibility, and the lateral orientation of the KTS/RTS tripeptide, may underlay the structural basis of RTS/KTS disintegrins' selectivity and specificity for integrin $\alpha_1\beta_1$. We have design, cloned, and recombinantly expressed in soluble form a *Frankenstein* disintegrin in which the RGD motif of ocellatusin was substituted by the RTS tripeptide of jerdostatin. This construct, and an array of subsequent mutants, represent useful tools to investigate the structural basis of the integrin inhibitory potency and anti-angiogenesis activity of $\alpha_1\beta_1$-blocking disintegrins. Moreover, our results provide evidence, that the RTS/KTS short disintegrins have been recruited into the venom gland of Eurasian vipers independently from the canonical neofunctionalization pathway of the RGD disintegrins.

Poster # 12

SNAKE VENOMICS OF *CROTALUS TIGRIS.* EVOLUTIONARY CLUES FOR GENERATING A PAN-SPECIFIC ANTIVENOM AGAINST CROTALID TYPE II VENOMS

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The tiger ratttlesnake, *Crotalus tigris*, is a is a medium-sized ground-dwelling dessert pitviper, that ambushes much of its prey but also active forages small rodents and lizards, juveniles relying heavily on lizards and adults depending more on rodents. In addition, this small rattlesnake has been known to eat fairly large prey, including kangaroo rats, packrats, and even spiny lizards. This is based upon its venom's high lethality, rated the highest of all rattlesnake venoms (LD_{50} value for mice is 0.07 mg/kg intraperitoneal, 0.056 mg/kg intravenous, and 0.21 mg/kg subcutaneous). The comparatively low venom yield (6.4-11 mg dried venom) and short 4.0-4.6 mm fangs of *C. tigris* possibly prevent severe envenoming in adult humans. However, the clinical picture could be very more serious if the person bitten was a child or a slight build individual.

We report the proteomic and antivenomic characterization of *Crotalus tigris* venom. This venom exhibits the highest lethality for mice among rattlesnakes and the simplest toxin proteome reported to date. The venom proteome of *C.tigris* comprises 7-8 gene products from 6 toxin families; the presynaptic β-neurotoxic heterodimeric $PLA_{2,}$ Mojave toxin, and two serine proteinases comprise, 66 and 27% of the *C. tigris* toxin arsenal, whereas a VEGF-like protein, a CRISP molecule, a medium-sized disintegrin, and 1-2 PIII-SVMPs each represent 0.1-5% of the total venom proteome. This toxin profile, in particular, the low metalloproteinase content, the high concentration of Mojave toxin subunits and its high toxicity LD_{50} 0.05(i.v)-0.07(i.p) mg/g of mouse body weight, place a *C.tigris* venom in the type II class defined by Mackessy. The venom composition really explains the systemic neuro and myotoxic effects observed in envenomated animals. In addition, we found that venom lethality of *C.tigris* and other North American rattlesnake type II venoms correlates with the concentration of Mojave toxin A-subunit.

An experimental antiserum was raised in rabbits by subcutaneous injections of subletal amounts of a mixture of venoms from *C.d. terrificus, Crotalus simus and Crotalus lepidus lepidus.* The ability of the experimental antivenom to effectively immunodeplete proteins from the type II venoms of *C.tigris, Crotalus horridus , Crotalus oreganus helleri, Crotalus scutulatus scutulatus, and Sistrurus catenatus catenatus* indicated the feasibility of generating a pan-American anti-*Crotalus* type II antivenom, suggested by the identification of shared evolutionary trends among South and North American *Crotalus* species.

Poster # 13

DEVELOPMENT OF A STABLE ISOTOPE-TAG-BASED LABELING METHOD FOR THE RELATIVE QUANTIFICATION OF PROTEINS FROM SNAKE VENOMS

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A deep knowledge of the toxin composition and the immunological profile of venoms are central for developing polyspecific antivenoms exhibiting the broadest possible paraspecificity. Antivenomic techniques enable the interactions between venoms and antivenoms to be examined in detail, and if combined with functional assays of specific activity and followed up by clinical trials of effectiveness and safety, can be powerful tools with which to evaluate the suitability of current and new antivenoms [1,2]. The original antivenomics protocol was based on the immunodepletion of toxins upon incubation of whole venom with purified antivenom IgGs, followed by the addition of a secondary antibody or immobilized IgG-binding moiety, such as protein-A or protein-G. Antigen-antibody complexes immunodepleted from the reaction mixture contain the toxins against which antibodies in the antivenom are directed. By contrast, venom components that remain in the supernatant are those which failed to raise antibodies in the antivenom, or which triggered the production of low-affinity antibodies. These components can be easily identified by comparison of reverse-phase HPLC separation of the non-precipitated fraction with the HPLC pattern of the whole venom previously characterized by a venomic approach. This antivenomic approach can not be used for $F(ab')_2$ antivenoms, which are increasingly produced by many manufacturers. On the other hand, long separation times required for the HPLC analysis and the following off line identification, it is very time consuming. Further, co-elution of similar components makes it difficult to quantify all single toxins by their UV-chromatogram. The employment of LC-MS provides more selective signals, but because of matrix ion suppression effects it is more difficult to quantify with external standards. To overcome these difficulties, we developed a fast LC-MS method using new differential isotope coded tags to perform relative quantification of venom proteins and their immunoreactivity with antivenoms. This poster describes the synthesis of n-D_5-ethyl-nicotinic acid and n-D_0-ethyl-nicotinic acid, the labeling of venom proteins from *Naja nigricollis* using carbonyldiimidazole as activation reagent, and the LC-MS analysis of the labeled venoms. Furthermore it shows the comparison of LC-MS protein identification with off-line protein identification from *Naja nigricollis* venom, reported in [3].

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Poster # 14

SNAKE POPULATION VENOMICS AND ANTIVENOMICS OF *BOTHROPS ATROX*: PAEDOMORPHISM ALONG ITS TRANSAMAZONIAN DISPERSAL AND IMPLICATIONS OF GEOGRAPHIC VENOM VARIABILITY ON SNAKEBITE MANAGEMENT

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We describe two geographically differentiated venom phenotypes across the wide distribution range of *B. atrox*, from the Colombian Magdalena Medio Valley through Puerto Ayacucho and El Paují, in the Venezuelan States of Amazonas and Orinoquia, respectively, and São Bento in the Brazilian State of Maranhão. Colombian and Venezuelan venoms show an ontogenetic toxin profile phenotype whereas Brazilian venoms exhibit paedomorphic phenotypes. Venoms from each of the 16 localities sampled contain both population-specific toxins and proteins shared by neighboring *B. atrox* populations. Mapping the molecular similarity between conspecific populations onto a physical map of *B. atrox* range provide clues for tracing dispersal routes that account for the current biogeographic distribution of the species. The proteomic pattern is consistent with a model of southeast and southwest dispersal and allopatric fragmentation northern of the Amazon Basin, and trans-Amazonian expansion through the Andean Corridor and across the Amazon river between Monte Alegre and Santarém. An antivenomic approach applied to assess the efficacy towards *B. atrox* venoms of two antivenoms raised in Costa Rica and Brazil using *Bothrops* venoms different than *B. atrox* in the immunization mixtures showed that both antivenoms immunodepleted very efficiently the major toxins (PIII-SVMPs, serine proteinases, CRISP, LAO) of paedomorphic venoms from Puerto Ayacucho (Venezuelan Amazonia) through São Bento, but had impaired reactivity towards PLA_2 and P-I SVMP molecules abundantly present in ontogenetic venoms. The degree of immunodepletion achieved suggests that each of these antivenoms may be effective against envenomations by paedomorphic, and some ontogenetic, *B. atrox* venoms.

Poster # 15

IDENTIFICACIÓN DE MARCADORES MICROSATÉLITE MEDIANTE SECUENCIACIÓN MASIVA DE DNA.

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Los microsatélites (SSR) son secuencias de DNA que contienen repeticiones en tándem de 1 a 6 nucleótidos. Dado su alto nivel de polimorfismo y su modo de herencia de codominancia, son los marcadores más utilizados para el estudio de la estructura genética de las poblaciones. Sin embargo la identificación de nuevos microsatélites utilizando la metodología clásica, basada en la obtención de genotecas enriquecidas en estos motivos repetidos y su posterior secuenciación con el método de Sanger, es bastante laboriosa y tiene un coste económico importante. Las nuevas tecnologías de secuenciación masiva de DNA están remplazando gradualmente los métodos tradicionales puesto que reducen considerablemente tanto el tiempo como los costes de la identificación de nuevos microsatélites en organismos no modelo. En este póster presentamos los resultados de pirosecuenciación de muestras obtenidas según dos procedimientos: DNA enriquecido en secuencias microsatélite y DNA total, y los comparamos con los resultados obtenidos con la metodología tradicional que implica clonación y secuenciación. Los resultados obtenidos en los dos procedimientos de pirosecuenciación ponen de manifiesto que, en ambos casos, el número de microsatélites detectados es mucho mayor que el obtenido mediante el protocolo tradicional. Aunque la comparación de ambas metodologías de pirosecuenciación requiere un diseño de análisis específico, a priori, con los datos de los que disponemos, podemos concluir que los resultados obtenidos de la pirosecuenciación de DNA total, proporcionan mayor número y diversidad de regiones microsatélite candidatas a ser utilizadas como marcadores. Además, la secuenciación de DNA total proporciona la información de otras regiones que pueden ser utilizadas en posteriores análisis.

Poster # 16

SERVICIO DE SECUENCIACIÓN MASIVA DE DNA

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La Unidad de Genómica del Servicio Central de Soporte a la Investigación Experimental (SCSIE) ha puesto en funcionamiento el nuevo laboratorio de secuenciación masiva de DNA que tiene como finalidad ofrecer servicios de secuenciación genómica y transcriptómica a todos los grupos de investigación de la Universitat de València así como de otras instituciones públicas o privadas que lo requieran. El nuevo laboratorio está equipado con dos plataformas de secuenciación masiva, el equipo GS Junior (Roche) y los equipos SOLID4 y 5500XL de Life Technologies. Las dos plataformas utilizan metodologías de secuenciación diferentes, y generan resultados distintos en cuanto a longitud de lectura y número total de secuencias obtenidas. Con la plataforma GS Junior se obtienen, mediante pirosecuenciación, secuencias de 400 nucleótidos con un total de 100.000 lecturas por análisis, mientras que con SOLID4 y 5500XL se obtienen secuencias de 50-75 nucleótidos, mediante ligación de sondas fluorescentes con codificación dual. Además esta última metodología permite la obtención de un número de lecturas por análisis del orden de 1.400 millones, lo que convierte a la plataforma 5500XL en el equipamiento de secuenciación de nueva generación más avanzado del mercado siendo muy adecuada para los proyectos que requieren un análisis en profundidad del genoma o transcriptoma. Las diferentes características técnicas de las dos plataformas nos permiten ofertar a los usuarios del servicio un gran número de aplicaciones como: resecuenciación dirigida de regiones del genoma, secuenciación “de novo” de genomas pequeños, secuenciación de amplicones como por ejemplo genes asociados a cáncer, estudios de metagenómica, secuenciación de transcriptoma completo y secuenciación de smRNA.

En el laboratorio de secuenciación masiva del SCSIE se llevan a cabo todas las etapas de los proyectos de secuenciación, desde la preparación de las librerías de DNA o RNA, la secuenciación en las distintas plataformas y el posterior análisis bioinformático de los datos obtenidos.

Poster # 17

DEVELOPMENT AND VALIDATION OF MRM METHODS TO QUANTIFY PROTEIN ISOFORMS OF POLYPHENOL OXIDASE IN LOQUAT FRUITS

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Multiple reaction monitoring (MRM), in mass spectrometry, is emerging as a promising technique for the detection and quantification of protein biomarkers in complex biological samples[1]. The high sensitivity, specificity, accuracy, assay speed and high sample throughput, represent a preferred option in the analytical compared to Western blotting or enzyme assays for analysis of protein isoforms. MRM assays detecting and quantifying proteolytic peptides unique to particular isoforms, i.e. proteotypic peptides at isoform level. We have focused on Polyphenol Oxidase (PPO), a plant conspicuous enzyme encoded by multigene families in loquat (*Eriobotrya japonica* Lindl.)[2]. PPO is responsible for protection of plants from biotic stress and the enzymatic browning of fruits and vegetables, making them more attractive to seed dispersal agents but also being a major cause of important economical losses in agriculture and food industry. An adequate management of PPO at plant breeding level would maximize the benefits and minimize the disadvantages of this enzyme, but it would require a precise knowledge of the biological role played by each isoform in the plant. Thus, for the functional study of the PPOs we have cloned, characterized and overexpressed fragments of three PPO isoforms from loquat to develop MRM-based methods for the quantification of each isoform. The method was developed using an ion trap instrument and validated in a QQQ instrument. It resulted in the selection of two peptides for each isoform that can be monitored by at least three transitions.

Keywords: enzymatic browning, fruit, isoform, MRM, polyphenol oxidase.

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Poster # 18

TRANSCRIPTOMIC ANALYSIS OF PSEUDOMONAS AERUGINOSA USING T3SS INDUCTION CONDITIONS

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The Gram-negative bacterium *Pseudomonas aeruginosa* is a major cause of health-care associated infections, including pneumonia and infections involving the urinary tract, wounds, burns and the bloodstream. Like other Gram-negative bacteria, *P. aeruginosa* interferes in eukaryotic host cells development by using a type III secretion system (T3SS). The contact with host cells allows bacteria the injection of toxic proteins, called exotoxines or effectors, directly into the cytosol of infected cells by using T3SS. Here we report the transcriptome analysis of this bacteria using the induction of this type of system under calcium-limited growth conditions.

Prokaryotic transcriptomics is a powerful tool for understanding gene regulation, physiology and pathogenicity in bacteria. After RNA extraction and mRNA enrichment, the sequencing resolution have been done using one of the next generation sequencing technologies (NGS) used for the whole transcriptome analysis, RNA-Seq. Solexa-Illumina is one of the three major sequencing platforms to produce the raw data into the fastq files subsequently used to compute differential expression between each two different conditions. Specifically in bacteria, we also have described the operon distribution above the transcriptome, throught the selection of all neighborhood genes expressed in the same strand with similarity in coverage into both genes and also into the intergenic sequence (IGS).

The results will summarize the transcriptomic organization of *P. aeruginosa PA14* and differential expressed genes identified after comparing two different conditions, with and without induction of the T3SS system, using an algorithm, NOISeq, that certifies the statistical significance.

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Poster # 19

PLATAFORMA DE PROTEÓMICA DE LA UNIVERSITAT DE VALÈNCIA

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La Proteómica es una disciplina científica relativamente reciente cuyo objetivo es el estudio de los proteomas. Comparado con el genoma, un proteoma representa un nivel superior de complejidad por su dinamismo y variabilidad. Si bien el genoma es único para una especie, el proteoma depende del tipo celular y su relación con el entorno en el que se encuentra, variando rápidamente en respuesta a su interacción con el medio. Entre estas variaciones se encuentran tanto cambios en las concentraciones de las proteínas como en los niveles de las modificaciones postraduccionales. Esta complejidad y dinamismo requieren el empleo de técnicas analíticas de gran precisión, sensibilidad y alto rendimiento que permitan la identificación, cuantificación y caracterización de un gran número de proteínas, por ello la base tecnológica de la proteómica actual es la espectrometría de masas. La relación entre ambas es tal, que los nuevos desarrollos tecnológicos en espectrometría de masas condicionan en gran medida los avances de la proteómica. La instrumentación necesaria tiene un elevado coste y requiere personal altamente cualificado. Por ello, la mayor parte de los investigadores acceden a las técnicas de proteómica a través de servicios centrales financiados por universidades y centros de investigación.

La sección de proteómica de la Universitat de València se creó con el objeto de ofrecer a la comunidad científica una infraestructura tecnológica lo más completa posible en el campo de la proteómica. En este sentido, la Universitat de València ha dotado a la sección de Proteómica con tecnología de última generación en espectrometría de masas, así como del personal necesario, para poder ofrecer a los investigadores los nuevos avances en proteómica. Como consecuencia de ello, la identificación y caracterización de proteínas se puede realizar con una mayor sensibilidad y eficiencia que aumenta considerablemente la cobertura de los proteomas en estudio. En consecuencia, los estudios de expresión diferencial de proteínas también son más resolutivos y potentes. Con la nueva instrumentación se puede abordar experimentos de *targeted proteomics* y cuantificación absoluta lo que ha permitido a la Universitat de València participar en proyectos como el del Proteoma Humano, auspiciado por HUPO. Una de las nuevas aplicaciones proteómicas que está despertando mucho interés y que se podrá implementar en la Plataforma de Proteómica es la obtención de imágenes mediante MALDI-TOF MS. En esta técnica se obtienen espectros de masas directamente de preparaciones histológicas con lo que se pueden obtener posteriormente una imagen del tejido para cada masa. De esta manera se puede estudiar la distribución de diversas moléculas en el tejido. Esta aproximación se está empleando mucho en el descubrimiento de biomarcadores.

Además de las mejoras tecnológicas, también se ha dotado a la sección de Proteómica de instrumentos bioinformáticos para analizar e integrar los datos generados lo que permitirá a los investigadores obtener la mayor información posible de los experimentos de proteómica.

Poster # 20

PLOIDY-LEVEL EFFECTS ON THE CLEMENTINE TRANSCRIPTOME

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Polyploidy has long been recognized as an important force in the evolution of flowering plants as often results in novel phenotypes that are not present in their diploid progenitors or exceed the range of the contributing species. The new acquired phenotypes may have an advantage in adaptation that may also enhance their interest for agriculture. Many crops, including citrus, are bred to a higher level of ploidy in search of desirable traits.

In previous works of our group synthetic clementines with different levels of ploidy were generated, specifically haploid, dihaploid and autotetraploid lines were obtained. This plant material represents a valuable tool for studying the effects of polyploidization at a molecular level since important traits are expressed in a different manner. Interestingly, dihaploid lines have been not able to flower, while haploid, diploid and autotetraploid lines have.

By using a microarray approach, we have accomplished a comprehensive analysis of transcriptome divergence among the newly created clementine lineages with different genome dosage and specifically have explored new gene expression patterns related to the ability to flower. Differences in expression in the bark tissue of the four lineages were found significant for ninety four genes. Clustering analysis results and expression profile of these genes in the 4 compared lineages were used as additional criteria to select 16 genes putatively involved in the flowering differences found in the lineages.

To explore their potential involvement in the flowering event we have analyzed their expression in floral organs and the occurrence of differential expression in juvenile and adult bark tissues. Results and conclusions will be presented.

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Poster # 21

ANALYSIS OF *GIGYF2* IN BASQUE POPULATION AND SYSTEMATIC REVIEW OF PARKINSON-RELATED CODING VARIANTS

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The *GIGYF2* gene has been proposed as the gene responsible of the association of *PARK11* locus to Parkinson's disease (PD). Although this association has been controversial, some genetic and functional studies, as well as some animal models point to the true association of this gene to de disease. In this work, some variants of *GIGYF2* were found in Basque PD patients, including one new and many others previously described coding variants. With the intent to clarify the relationship between this gene and the disease, a systematic review was carried-out using the frequencies available for all the coding variants founded in PD genetics studies, including the new data obtained from this work. The potential pathogenicity of coding variants was also analyzed using bioinformatics methods. The results point to a lack of association of variants of this gene to the disease.

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Poster # 22

INFLAMACIÓN Y NEURODEGENERACIÓN: ANÁLISIS DE COL4A3BP

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La inflamación es un fenómeno central en la mayoría de las enfermedades neurodegenerativas, aunque no hay un consenso claro en cuanto a si está relacionada con la etiología o la evolución de dichas enfermedades. Además, se sabe que existen factores genéticos que influyen en la susceptibilidad para desarrollar enfermedades neurodegenerativas. Por lo tanto, cualquier gen que pueda afectar el estado inflamatorio en el cerebro de personas afectadas por enfermedades neurodegenerativas podría contener modificadores genéticos de riesgo para estas enfermedades. Por estas razones, hemos llevado a cabo el análisis de COL4A3BP como un gen candidato para enfermedades neurodegenerativas. COL4A3BP codifica para la proteína de unión a antígeno de Goodpasture (GPBP) que está implicada en la enfermedad autoinmune Goodpasture. GPBP se expresa en varias áreas del cerebro, especialmente en hipocampo, corteza cerebral y ganglios basales. En esta presentación se muestra el análisis de COL4A3BP en un grupo de enfermedades neurodegenerativas y neurológicas. Se secuenciaron 1000 pb upstream del inicio de traducción, región que contiene un exón que se expresa de manera específica de tejido, y se encontraron nuevas variantes que no mostraron asociación con ninguna de las enfermedades estudiadas.

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Poster # 23

MICROARRAYS ANALYSIS OF GLIOBLASTOMA MULTIFORME AND ANAPLASTIC ASTROCYTOMA. RELATIONSHIP BETWEEN GENE EXPRESSION AND DIAGNOSIS TUMOR.

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Purpose: Anaplastic astrocytoma (AA) and its more aggressive counterpart, glioblastoma multiforme (GBM), are the most common intrinsic brain tumors in adults and are almost universally fatal. A deeper understanding of the molecular relationship of these tumor types is necessary to derive insights into the diagnosis, prognosis and treatment of gliomas. Microarrays-based gene expression analysis can be used to classify tumors according to histological subtypes and to identify differentially expressed genes between these tumor types. In this communication, we show differences in genomic profiles based on microarrays analysis of 20 biopsies for the distinction between classes of tumor.

Experimental Design: 20 human biopsies (15 GBM and 5 AA) were obtained from 20 patients at the Department of Neurosurgery of the Clinical University Hospital of Valencia. We have studied 15 cases of GBM and 5 cases of AA from patients by microarray-based gene expression analysis.

Results: Principal Component Analysis (PCA) show difference between GBM and AA for unsupervised PC3 (three components or the third component). We can be observed a deviation for some samples that not have a correlation with their histopathologies profiles.

Conclusion: We have performed a microarrays analysis of genome-scale mRNA expression data for 20 human malignant gliomas and have identified a list of > 300 probe sets that are significantly different between GBM and AA. These feature lists could be utilized to aid in diagnosis, prognosis and grade reduction of high-grade gliomas and to identify genes that were not previously suspected of playing an important role in glioma biology. More generally, this approach suggest that combined analysis of existing data sets can reveal new insights and that the large amount of publicly avalaible cancer data sets should be further utilized in a similar manner.

Poster # 24

METABOLIC SUBGROUPS IN HIGH-GRADE GLIOMA SHOW CORRELATION TO ANGIOGENESIS PATHWAYS

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Objective: The metabolic consequences of different angiogenesis gene expression profiles in gliomas are still unknown. Gene expression profiling of different metabolic phenotypes of high grade glioma may provide new information for better management of this disease. We show high grade glioma NMR molecular profiles correlated with gene expression profiles of 30 high-grade glioma biopsies.

Method: Forty samples of human glioma tissue (23 GBM and 7 AA) were obtained and characterized at the Clinic Hospital of Valencia. The GeneChip Human Gene 1.0 ST Array was used for gene expression analysis. Metabolic profiling of the samples was performed on frozen tissue by nuclear magnetic resonance.

Results: Metabolic profiling revealed two major subgroups within samples with histopathological diagnosis of GBM. The phospholipids pattern, the glycolytic ratio and the glutamine/glutamate metabolic are the major differences between subgroups. Microarrays gene expression analysis revealed more than 300 probesets significantly different between these metabolic subgroups.

Conclusion: There is a correlation between genes involved in angiogenesis and metabolic profiles. This correlation involves metabolites closely related to higher glycolytic rate, to proliferation and to hypoxia.

Poster # 25

MOLECULAR SUBGROUPS OF BENIGN MENINGIOMA WITH DIFFERENT BIOCHEMICAL AGGRESSIVENESS

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Objective: Sometimes, meningiomas with benign histological diagnosis tend to recur. Better classification of meningiomas will improve clinical decisions in these tumors. We obtained metabolic and gene expression profiles on 30 benign and 10 atypical meningiomas.

Method: Tumor samples were obtained at the Clinic Hospital of Valencia and categorized by WHO classification. Karyotypic analyses were performed according to ISCN (1995). The GeneChip Human Genome U133 plus 2.0 Array was used for gene expression analysis. Metabolic profiling of the samples was performed on frozen tissue by Nuclear Magnetic Resonance.

Results: For most of the metabolites, the values for benign meningioma with chromosomal instabilities were closer to atypical meningioma than to chromosomally normal benign meningioma. Most of the metabolic changes observed in benign meningioma with chromosomal instabilities reflect higher tumor metabolism. Gene expression profiling shows main differences in genes involved in the regulation of transcription.

Conclusion: The metabolic and gene expression profiles allow us detecting biochemical aggressiveness in otherwise benign tumors. The methodology used in this study may also open new possibilities in the diagnosis of meningioma.

Poster # 26

EL GENOMA DE *RHIZOBIUM RHIZOGENES* K84 DESVELA ALGUNOS SECRETOS DE LA VIDA EN LA RIZOSFERA

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La cepa K84 de *Rhizobium rhizogenes* es un eficaz agente de control biológico a nivel mundial de los tumores de plantas causados por diversas especies de *Agrobacterium*, enfermedad conocida en inglés como "crown gall". La cepa K84 es una buena colonizadora de las raíces de diversas especies de plantas, y ha mostrado eficacia en el control biológico en gran cantidad de huéspedes, desde plantas herbáceas hasta diversos frutales. El análisis en profundidad de su genoma nos ha desvelado cuáles pueden ser las adaptaciones que la convierten en una bacteria rizosférica modelo de amplio espectro. Así, su genoma codifica gran cantidad de proteínas quimiorreceptoras, lo que sugiere un estilo de vida complejo, capaz de tactismo hacia gran cantidad de compuestos que pueden estar presentes en los exudados de raíces. También posee un gran repertorio de sistemas de transporte, lo que refleja su versatilidad metabólica para utilizar los compuestos exudados por las plantas. Más interesante es el destacado número de genes reguladores, incluyendo abundantes sistemas de regulación de dos componentes, lo que indica una gran plasticidad para modular su expresión génica y adaptarse rápidamente a estímulos cambiantes en el suelo y en la rizosfera. También posee genes de desarrollo polar y adhesinas que podrían contribuir al reconocimiento, unión y colonización de las raíces. Asimismo, contiene un elevado número de genes que codifican diversos polisacáridos que podrían también contribuir a la colonización y supervivencia en la rizosfera. El genoma de K84 codifica diversos sistemas de secreción tipo I, tipo II y sorprendentemente un sistema de secreción de tipo 3 (T3SS). También presenta tres sistemas completos de secreción de tipo IV. En conjunto, estos sistemas de secreción podrían, no sólo mediar la interacción con la planta, sino también con otros organismos en la rizosfera. Por último, se han identificado diversos genes relacionados con el desarrollo de las biopelículas que la cepa K84 forma durante la colonización del huésped. La continuación del análisis en detalle de éste y otros genomas completos de bacterias rizosféricas, aportará, sin duda, más luz sobre la adaptación a la vida en la rizosfera y contribuirá a desvelar algunos de los muchos secretos de las interacciones bacteria-planta.

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Poster # 27

EXOME CHARACTERIZATION OF GENE VARIANTS RELATED TO THE FERTILITY STATUS OF DIFFERENT HUMAN POPULATIONS

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Genome research has been revolutionized by Next-Generation Sequencing (NGS) technologies. From this perspective, Biomedicine is increasingly using them, and in particular the exome analysis, to associate genetic variants with phenotypes. Endometrial receptivity phenotype is the stage in the menstrual cycle when the embryo implantation may occur. Research regarding human reproductive health is a matter of interest in human society, however it has not yet been studied deeply using these revolutionary approaches.

The main objective of this study was to characterize the exome variants of endometrial receptivity phenotype genes in control human populations. We analyzed the exome of samples from 14 populations of the latest *1000 Genomes* release [1] and the Andalusian population from the Medical Genome Project [2]. In order to define the normal endometrial receptivity variant profile, each population has been characterized and compared within the others.

These genes were characterized and analyzed using biological databases and Babelomics platform [3]. A functional enrichment and a network gene analysis were performed to study the gene biological system. Finally, we have investigated this fertility status under a Systems Biology perspective, establishing the relationship between genetic variants and topological parameters of this endometrial receptivity network.

[1] The 1000 Genomes Project Consortium. *Nature* **467** (2010) 1061-73.
[2] Medical Genome Project http://www.medicalgenomeproject.com/
[3] Medina et al. *Nucleic Acids Res.*(2010) W210-3.

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Poster # 28

TRANSCRIPTOMIC DIFFERENCES BETWEEN GROWTH RATE CLASSES UNCOVERED BY MICROARRAYS IN THE CLAM *RUDITAPES DECUSSATUS*

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Growth rate is one of the most important commercial traits in cultured molluscs. However, knowledge of the molecular basis of growth differences in this group of animals is scarce. A genetic basis is inferred from artificial selection experiments with positive response, from heritability studies and from the frequent observation of correlations between heterozyygosity at protein coding genes and growth in wild populations. Physiological studies indicate that proteins coded by genes expressed in the nervous ganglia can have a role as endocrine regulators of growth.

Transcriptomic studies may be a powerful way to increase our knowledge of molluscan growth genetics and molecular biology. Massive parallel sequencing of the transcriptome in oyster families exhibiting heterosis for growth showed that genes expressed in an overdominant way were concentrated in protein metabolism and energy production routes in larvae. This suggests that detailed examination of tissues for transcriptomic differences between fast and slow growers might help to understand the biology of adult mollusc growth.

Here we report on an experiment designed to explore this topic in the clam *R. decussatus*, an infaunal bivalve species that is commercially important in southern Europe. *R. decussatus* has a slower growth rate than other clams of the subfamily Tapetinae. This can hamper clam production in some regions by extending the grow out period to more than 3 years and, consequently, increasing the production costs and the risk of production loss due to adverse environmental conditions.

The growth of 40 individual clams taken from the wild population of Ria Formosa (Portugal) was followed during a 3 month period in captivity. Shell length and height was measured at the onset and at the end of the experiment, and relative growth was estimated from these values by regression analysis, correcting for the initial size. RNA extracted from the gills of the 5 fastest and the 5 slowest growing clams was used for individual microarray hybridization. After normalization, 110 genes showed significant differences between the two groups, as indicated by Student t tests performed with Bonferroni correction. Functional analysis with DAVID indicated that this gene set was enriched for genes related to protein synthesis and energetic metabolism.

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Poster # 29

UNRAVELING THE TRANSCRIPTIONAL PROGRAM IN THE TRANSDIFFERENTIATION OF MELANOMA TO ADIPOCYTE-LIKE CELLS.

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During transdifferentiation, cells regress to a point where they can switch lineages, allowing them to differentiate into another cell type. This process is driving by a downregulation of one genetic programme and a upregulation of the new genetic programme. Transdifferentiation has been observed to occur naturally in the regenerating lens of the newt. Moreover, literature reports some examples of experimental induction of transdifferentiation, describing the transcription and epigenetic remodelling factors that drive the process.

Recently studies have characterized the transdifferentiation of human cancer cells into adipocyte-like cells, induced by the presence of specific unsaturated fatty acids. In this project, a RNA-seq approach has been applied to the study of the transdifferentiation of melanoma MALME-3M. A set of data from different states of this process (time from incubation with unsaturated fatty acids: 0 h, 3 h, 7 h, 11 h, 15 h and 24 h) is available.

The bioinformatic analysis of this data has allowed us the quantification of the changing expression levels of each gene, first step for obtaining relevant biological information. Based on the analysis of these expression levels, we present the preliminary results, describing mechanisms and genes that could be relevant in the transdifferentation process. We have observed an activation of the chromatin remodelling processes that include DNA methylation, histone deacetylation and local chromatin structure alterations. In this context, several genes are implicated: DNMT1, HDAC3, HDAC9, JMJD1C and HMGA2. Another significant pathway to consider is cell cycle regulation. We have observed an initial activation of cell cycle followed by a progressive downregulation. We point out that RB1 could have an important role in this regulation of cell cycle. Furthermore, analysis of the gene expression reveals important implications of non-coding genes, lincRNA and antisense, and that they maintain regulatory interactions with other genes.

Poster # 30

ANALYSIS OF THE IMMUNE SYSTEM PROTEIN IN ORAL AND INTRAPERITONEAL *TRYPANOSOMA CRUZI* INFECTION TRIGGERED BY *CL* AND *Y* STRAINS

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Several studies have demonstrated a high capacity of infection by metacyclic trypomastigotes with majority vector prevalence in some regions. *Trypanosoma cruzi* appears to regulate protein expression primarily posttranscriptionally through variations in mRNA stability or the translational efficiency of mRNAs. This limits the use of DNA microarrays and makes proteomic analysis especially attractive for examining global changes in protein expression during development in *T. cruzi*. Oral infection has caused frequent outbreaks of Chagas' disease arising from the ingestion of contaminated food. However little to nothing is known about the immunopathology comparing these two different routes of infection with strains of high (*Y*) and low virulence (*CL*). The aim of this study was to assess of concentrations immune system protein, like IFN-γ and IL-10 produced in Wistar rats infected orally and intraperitoneally with different strains of *T. cruzi*, *Y* and *CL*. Wistar rats were intraperitoneally infected (I.P.) ($1x10^5$; *n*=10) and orally infected (IO) ($8x10^5$; *n*=10) with *Y* and *CL* strains of *T. cruzi*. Metacyclic trypomastigotes infctive forms was used. Immune protein (interleukin) were measured in the serum of these animals at the peak of the infection for each strain. For determination of IFN-γ and IL-10, the R&D Systems kit (Minneapolis,MN, USA) was used. All samples were analyzed separately twice. The (I.P) infection with the *Y* strain showed a higher concentration of INF-γ when compared with (I.P) infected with CL strain (*P* <0.05). On the other hand the when analysed animals (I.P) infected with Y strain showed an decrease of IL-10 compared to the group with *CL* strain, showed no statistical. The IO groups with the *CL* strain triggered increased concentrations of INF-γ, unlike animals infected orally with the *Y* strain (*P* <0.01). The same resulted not was observed about immune protein Th-2 type, because the IO with *CL* showed decrease of IL-10 when compared with animals infected with Y strain (*P* <0.05). In (I.P), *Y* strain produced an immune response of the Th-1 type, with IFN-γ level more elevated when compared with *CL* strain. IL-10 level in (I.P) showed no statistical difference between the *Y* and *CL* strains. In the process of infection (I.P) with strain *Y*, specific receptors of Th-1 type immune cells directly interacted with the parasite and were able to promote an acute immune response faster, due to the high virulence and rapid intracellular replication of the parasite. In case of IO with *CL* strain, a significant increase of IFN-γ was detected when compared to strain Y. When considering IL-10 level in case of IO, *CL* strain had a concentration more elevated than the *Y* strain. IO with *CL* strain induces a Th-1 response which overrides the immune response caused by the *Y* strain. This ability is due to higher resistance of the *CL* strain to host immune system during the first stage of gastric infection and too be able to have that same characteristic in the blood system.

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Poster # 31

MELATONIN PREVENTS HEART DAMAGE DURING THE CHRONIC PHASE OF CHAGAS' DISEASE BY INHIBITING IMMUNE SYSTEM PRÓ-INFLAMMATORY PROTEINS

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The inflammatory answer caused by the infection with *Trypanosoma cruzi* plays an important role for the host, with respect to both resistance against the infection as well as the evolution of the Chagas' disease chronic phase. Cardiac parasitism was apparently related to the increased expression of cytokines and chemokines, and increased expression of desmin and other structural proteins as suggested by proteomic analysis. The plasmatic levels of the biochemical marker creatine kinase-MB (CK-MB) allow to measure the severeness of the lesions of the myocardial muscle. In this context, the immune system pro-inflamatory (cytokines TNF-α and IFN-γ) are proteins that play a double edge sword role in Chagas' disease. These proteins help to control the parasitemia and in the same time can trigger a exacerbated immunological-inflammatory answer. Melatonin is a neuro-hormone, derived from the aminoacid tryptophan which is being produced mainly by the gland Pineal. It (melatonin) displays immunomodulatory and anti-inflammatory properties. We evaluated the effects of Melatonin on the chronic cardiac inflammatory process of the Chagas' disease. This was done by analyzing the levels of CK-MB, TNF-α and IFN-γ of Wistar rats infected by $3x10^5$ forms of the *Y* strain *T. cruzi*. We worked in groups consisting of 5 male Wistar rats each, with a weight of 250g each individual. They were divided as follows: Not infected control (NIC), Not infected and treated with Melatonin (NITM), infected control (IC), infected and treated with Melatonin (ITM). After a span of 60 days post-infection, the animals were treated orally with a solution containing Melatonin dissolved in polyethylene glycol 400 and distilled water 1:1. The same experiments were undertaken 90, 120 and 180 days after infection. Then the rats were decapitated after being anesthetized. The concentrations of TNF-α and IFN-γ was determined examination cardiac tissue samples and CK-MB was dosed of serum of the animals, using specific kits. The animals treated with melatonin showed a significant reduction in the concentration of inflammatory mediators and plasmatic levels of CK-MB-*$p<0,05$. Considering the reduction of the inflammatory process and cardiac damage, the above results suggest a protective effect of Melatonin.

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Poster # 32

NATURAL TRANSMISSION OF FASCIOLIASIS & IDENTIFICATION OF THE INTRAMOLLUSCAN TREMATODE LARVAL STAGE, CONFIRMED BY NUCLEAR rDNA AND mtDNA SEQUENCING IN ARGENTINA

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Fascioliasis is widespread in livestock in Argentina with important endemic areas. It not only affects cattle, but also sheep and goats, particularly in the arid, marginal regions where these animals can be the only means of subsistence. There are reports of human cases and the proximity to endemic regiqns in Chile and Bolivia is cause for concern. Mendoza, an Andean province in Midwestern Argentina is currently making efforts for its control due to the very high prevalence reported in animals. Traditionally Lymnaea viator was considered the only vector in the region, but its identification was based solely on morphological criteria. Lymnaeid snails have great intraspecific variability regarding shell morphology and uniformity of their anatomy, thus, these characteristics do not suffice for correct species identification. Sequences of molecular markers amongst the nuclear ribosomal DNA and the mitochondrial DNA appear to be the most useful tools for specimen classification purposes. In a long term initiative to evaluate which are the fascioliasis areas of most concern, studies were performed in a recreational farm in Mendoza located at the foot of the Andes which included: a) coprological studies of cattle, goats, horses, donkeys and a llama to detect *Fasciola hepatica* eggs by the rapid sedimentation technique b) classification of the lymnaeid vector present and c) verification of natural transmission of fascioliasis by identification of the intramolluscan trematode larval stages. Lymnaeid and trematode classification was verified by means of nuclear ribosomal DNA and mitochondrial DNA marker sequencing. Coprological studies showed that all mammal species of the farm were affected by *F. hepatica* infection: 3 infected of 4 cattle analyzed, 3 of 4 goats, 3 of 7 horses, 4 of 4 donkeys, and 1 out of 1 llama. Complete sequences of 18S rRNA gene and rDNA ITS-2 and ITS-1; and a fragment of the mtDNA cox1 gene demonstrate that the lymnaeid belongs to the species *Lymnaea neotropica*. Redial larval stages found in a *L. neotropica* specimen were ascribed to *F. hepatica* after analysis of the complete ITS-1 sequence. The finding of naturally infected *L. neotropica* is the first of this lymnaeid species not only in Argentina but also in Southern Cone countries. Its presence in relation to so many species of animals with *F. hepatica* together with the recent report of *Galba truncatula* in the same province, adds to the complexity of the epidemiology of fascioliasis transmission in the region.

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Poster # 33

rDNA AND mtDNA ANALYSIS OF THE FRESHWATER LYMNAEID SNAIL SPECIES *LYMNAEA VIATOR* AND *GALBA TRUNCATULA*, THE MAIN FASCIOLIASIS VECTORS IN CHILE

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Freshwater lymnaeid snails transmit fascioliasis, a highly pathogenic liver parasitosis caused by trematode species of the genus *Fasciola* which affects humans and livestock species in all continents. In South America, *Fasciola hepatica* infection poses serious health problems in both humans and livestock. Molecular studies were undertaken to clarify the specific status of the lymnaeids, their geographical distribution, and fascioliasis transmission capacity in different regions of Chile. In this country, the medical impact appears yearly stable and mainly concentrated in central regions, where the veterinary problem is highlighted by higher animal prevalences. Analyses were performed by rDNA ITS-2 and ITS-1 and mtDNA *cox*1 sequencing and comparison with neighbouring and other American countries, as well as with countries of other continents when needed. Results obtained showed that the lymnaeid fauna of mainland Chile is relatively poor, including only two authochthonous species, *Lymnaea viator* and *Pectinidens diaphana*, and a third introduced species of Palaearctic origin *Galba truncatula*. Both *Lymnaea lebruni* and *Lymnaea patagonica* proved to be synonyms of *P. diaphana*. In this country, *Galba truncatula* appears to have always been confused with *L. viator* and seems distributed from Región VI to Región IX, overlapping with human endemic areas. DNA sequencing results suggest that the absence of correlation between remote sensing data and disease prevalences could be due to transmission capacity differences between *L. viator* and *G. truncatula*. Results furnish a new baseline on which to undertake future appropriate studies on transmission, epidemiology and control of this important disease in Chile.

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Poster # 34

MARCADORES DE ADN RIBOSOMAL Y MITOCONDRIAL: VENTAJAS E INCONVENIENTES EN ESTUDIOS SOBRE LA EVOLUCIÓN DE PARÁSITOS Y VECTORES
(RIBOSOMAL AND MITOCHONDRIAL DNA MARKERS: ADVANTAGES AND DISADVANTAGES IN STUDIES ON THE EVOLUTION OF PARASITES AND VECTORS)

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Las técnicas moleculares están mostrando que las enfermedades parasitarias son mucho más complejas de lo que se pensaba. Así, la gran heterogeneidad genética subyacente en parásitos, vectores u hospedadores intermediarios y hospedadores definitivos, vislumbra un amplísimo abanico de diferentes interacciones entre los diferentes compartimentos en cada enfermedad. Los métodos y técnicas moleculares permiten profundizar en la transmisión de las enfermedades hasta dilucidar diferentes circulaciones, a través de diferentes hospedadores intermediarios/vectores y hospedadores definitivos y reservorios, y los requerimientos medio-ambientales de las fases de vida libre de los parásitos y de sus fases parásitas en invertebrados transmisores y así comprender las diferentes situaciones epidemiológicas, patrones de transmisión y vías de contaminación en las diferentes zonas geográficas y épocas del año. Estos conocimientos resultan indispensables para establecer las medidas de control que, debido a la heterogeneidad, en muchos casos deberán diferir de un lugar a otro. Dos buenos ejemplos de esta complejidad genética, lo constituyen dos enfermedades parasitarias bien distintas pero ambas de transmisión indirecta y de carácter zoonótico, como son la Enfermedad de Chagas y la Fascioliasis. Dada la importancia de los vectores de estas dos enfermedades (triatominos en la Enfermedad de Chagas y lymneaidos en la Fascioliasis) se entiende el gran número de estudios mediante secuencias de ADN ribosomal nuclear y ADN mitocondrial recientemente publicados. Sin embargo, resultados y conclusiones discrepantes en diferentes publicaciones sugieren la necesidad de unificar criterios para evitar la posibilidad de una creciente situación de confusión. Con este fin, se ha llevado a cabo un análisis comparado de la eficiencia, peso de las diferentes características, limitaciones y problemas de cada uno de lo marcadores de ADN a la luz de resultados obtenidos en estudios de poblaciones, híbridos, subespecies y especies de triatominos y lymnaeidos, con base en conocimientos sobre estos marcadores moleculares en general. La necesidad de tener en cuenta las tasas evolutivas comparadas de los diferentes marcadores del ADN ribosomal y ADN mitocondrial se muestra crucial a la hora tanto de seleccionar un marcador según la pretensión del estudio como de interpretar correctamente los resultados obtenidos. Además, el incremento de estudios de esta índole indica la conveniencia de seguir una nomenclatura unificada simple sobre códigos de haplotipos combinados en parásitos y vectores.

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Poster # 35

CHANGES IN GENE EXPRESSION FOLLOWING AN ACUTE TOMATO INTAKE. A RANDOMIZED CROSS-OVER FEEDING CLINICAL TRIAL

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Tomato intake could act through still unknown epigenetic mechanisms influencing gene expression whose individual or joint actions could explain the beneficial effects that tomato has on reducing several pro-inflammatory markers. However, currently, the genes that are down- or up-regulated after tomato intake are unknown. Thus our aim was to identify these genes using both a candidate gene selection approach and a whole genome screening.

We performed a randomized feeding clinical trial (n=40 healthy subjects) who receive three interventions (raw tomato, tomato sauce and tomato sauce with oil). Participants were instructed to abstain from tomato and polyphenol rich foods before the intervention. Blood samples were collected at several times and RNA was extracted. To study gene expression, we pre-selected some candidate genes related to inflammation and oxidative stress and also undertook a screening by using a whole human transcript microarray. All selected genes were analyzed by RT-PCR. In the overall pathway analysis using the microarray, we detected that after tomato intake, the most enriched were genes involved in: 1) the regulation of transcription, 2) in DNA-binding and 3) in nucleosome, suggesting epigenetic mechanisms. In the individual gene expression analyses the most remarkable results were obtained for the following genes: SOD1, CAT, GPX3 and MMP9 (candidate-selected genes) and for ARRDC3, DDIT4, DUSP and GDI2 (microarray-validated genes).

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Poster # 36

NANONUTRIGENÓMICA COMPUTACIONAL: PROPUESTA PARA LA APLICACIÓN DE LA NANOINFORMÁTICA EN LA CARACTERIZACIÓN DE INTERACCIONES DE NANOCOMPONENTES CON FACTORES GENÉTICOS Y AMBIENTALES POR MEDIO DE UNA ONTOLOGÍA EN LA INVESTIGACIÓN EN NUTRIGENÓMICA

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La Nanotecnología estudia los nanomateriales a nanoescala, a nivel de átomos y moléculas, descubriendo nuevas propiedades hasta hoy desconocidas, para crear e implantar nanodispositivos o nanosistemas. Maojo et al. aseguran que la informática será clave en la investigación médica de las próximas décadas [1]. Por ejemplo, existen algunas nanopartículas y nanodispositivos aprobados o en revisión por la United States Food and Drug Administration (FDA) [2], por lo cual, se está planteando la regulación de las nanotecnologías sanitarias [3]. Así pues, la Nanoinformática es una disciplina emergente que pretende recolectar, organizar y publicar la gran cantidad de información generada por la Nanotecnología y, en particular, por la Nanomedicina. La Nanomedicina también está en su fase incipiente y está previsto su crecimiento de forma exponencial en los próximos años, sobre todo en la organización de la información generada [4, 5].

Este trabajo se presenta como un subproyecto en el marco de un proyecto de tesis doctoral cuyo objetivo principal es desarrollar un sistema computacional nanomédico que integre nuevos modelos conceptuales, arquitecturas de información y mecanismos computacionales específicos de la Nanoinformática y la Nanomedicina. Se pretende disponer de un modelo de datos de nanocomponentes unificado, centralizado, verificado y estandarizado, para comparar su caracterización de una forma inteligente acelerando el proceso de desarrollo de la investigación nanotecnológica y nanomédica. Este modelo se aplicará a la regulación de la ingestión de alimentos y de otros procesos metabólicos implicados en el desarrollo de la obesidad o en distintos fenotipos de las enfermedades cardiovasculares. Este objetivo principal se desglosa en varios objetivos específicos, uno de los cuales es el de este subproyecto: definición de una ontología nanotecnológica aplicada a la Nanonutrigenómica.

Proponemos el concepto de Nanonutrigenómica como una nueva disciplina derivada de la Nutrigenómica que estudia la intervención de los nanocomponentes en nutrición y sus efectos sobre el organismo humano identificando y caracterizando los fenotipos intermedios y finales. Para ello, la Nanoinformática debe aplicarse en recopilar la información mínima y suficiente que permita poder identificar de forma única los nanomateriales conocidos; tiene que definir los tipos de relaciones existentes entre los nanocomponentes; y debe analizar esta información y modelar o simular nuevas relaciones entre estos para poder predecir las consecuencias de las interacciones y toxicidad de los nanocomponentes una vez administrados. Todo ello pasa por disponer de información estructurada en el ámbito de la Nanomedicina y la Nutrigenómica, a partir de la cual se realizarían búsquedas avanzadas de forma sencilla. Tecnológicamente, esto se puede conseguir diseñando y construyendo una ontología que recoja los términos necesarios en el ámbito de los nanocomponentes nutrigenómicos.

Por tanto, el subproyecto planteado consiste en las siguientes fases: 1) Definición del conjunto mínimo de datos para la caracterización de los nanomateriales y nanocomponentes; 2) Definición de un lenguaje formal, basado en XML, para la caracterización de los nanocomponentes, las relaciones entre los mismos y entre nanocomponentes y otros factores, como los genéticos y ambientales (dieta, estilo de vida, consumo de alcohol y tabaco, etc.); 3) Definición de mecanismos para la simulación de interacciones de los nanocomponentes con factores genéticos y ambientales; 4) diseño de una ontología de nanocomponentes nutrigenómicos que recoja el conocimiento relativo al conjunto mínimo de datos, las relaciones entre nanocomponentes y las interacciones entre nanocomponentes y factores genéticos y ambientales; 5) construcción de la ontología de nanocomponentes nutrigenómicos; 6) integración conceptual y lógica de la ontología, los mecanismos de simulación de interacciones y el lenguaje de definición de los nanocomponentes nutrigenómicos; y 7) diseño y construcción de una plataforma computacional operativa que soporte la integración de la Fase 6 y proporcione una interfaz de usuario amigable y flexible.

El proyecto de tesis está en curso de realización y, por tanto, el trabajo presentado es una de las propuestas que forman el conjunto de subproyectos a desarrollar para la consecución de la tesis doctoral del primer autor, para la discusión de la comunidad científica y obtención de sugerencias y críticas que mejoren dicha propuesta.

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Poster # 37

INSIGHTS ON MITOCHONDRIAL GENOTYPING USING ION TORRENT

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Ion Torrent PGM is the fastest and the cheapest benchtop sequencer up to date. The ability to use a combination of different capacity chips (314, 316 and 318), depending on the amount of sequencing required for the project, together with the use of molecular identifiers also makes Ion Torrent one of the most versatile sequencer on the market, easy to be applicable to different research and clinical areas.

However there is still a lot of way to run in the implementation of new protocols and improvements at different stages of the sequencing procedure in order to make better profit of the throughput provide by the sequencer.

One critical step in order to obtain high quality results is to have high quality DNA prior the library preparation protocol. Here we present a new method for whole human mitochondrial DNA (mitDNA) genome sequencing where we have implemented a combination of overlapping primers that in combination with different extraction methods allow us to discern the faster and more accurate methodology for human mitDNA analysis using PGM.

Our results indicate that Pitcher and co workers (Pitcher et al 1989) extraction method together with the mitDNA amplification resulted on the faster and cost efective method for mitDNA sequencing using Ion Torrent.

In a 100nt run we obtained 146378, 151655, 141872 and 106983 sequences for 4 different extraction methods in 4 different individuals respectively with a mean length of 106.69, 116.14, 122.05 and 98.43 (Pitcher method being the latest). Mapped reads against the reference genome resulted on a mean coverage of 703.11, 1037.58, 825.07 and 501.93, where the percentage of high quality mapped reads (>Q20) were 59.62%, 80.58%, 61.92% and 63.02% respectively, This indicates that if we take into account the percentage of sequences that are incorporated into the mapping step, method 2 was the best in percentage of sequences mapped from the total amount of sequences obtained for that sample. If we take the percentage of high quality bases incorporated into the mapping, the method that incorporates more bases into the mapping is the Pitcher method with a 77.94% of sequenced bases. Resulting at the end that all the methods have a 100% of the bases of the reference genome covered at 20x, and 99.994% at 100x, except method 3 that have 99.982%.

Take into account all the results; we can say that Pitcher DNA extraction method, prior amplification with the set of overlapping primers that we have developed, is the best among the 4 compared methods. On the one hand this method requires more wet-lab time prior to the amplification step, being this a point against the method; on the other hand, even that Pitcher method was the one with less sequences obtained during the sequencing step, the mitochondrial genome was better covered, indicating that we the contamination with human DNA is lower and we can make more profit of the obtained sequences. As a consequence we can conclude that we can include more samples in the same chip making it more cost effective.
