Societat Catalana de BIOLOGIA

XXII JORNADES DE BIOLOGIA MOLECULAR

Organitzades per la Secció de Biologia Molecular de la Societat Catalana de Biologia
amb la col·laboració de la Secció de Biologia i Indústria

INSTITUT D´ESTUDIS CATALANS

Carrer del Carme 47

Barcelona

2 i 3 de Juny de 2015

Coordinadors:

Secció Biologia Molecular de la SCB
Albert Jordan Vallès (IBMB-CSIC)

Secció de Biologia i Indústria de la SCB
Ramon Roca Puig

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Dimarts 2 de juny

8:30-9:00 Recollida documentació

9:00 Benvinguda del coordinador de la Secció de Biologia Molecular

9:10-9:50 Conferenciant convidat

Lucas Carey
Universitat Pompeu Fabra (UPF)
Organisms may use temporary genetic variability to combat temporary environmental changes

Moderadors: Oriol Gallego (IRB), Bernat Crosas (IBMB-CSIC)

9:50-10:05
Control of gene expression by stress-activated protein kinases (SAPKs)
Carme Solé, Mariona Nadal, Francesc Posas & Eulalia de Nadal
Universitat Pompeu Fabra

10:05-10:20
The DEAD-box helicase Dhh1 promotes translation of highly structured mRNAs
J Jungfleisch[1], D Nedialkova[2], I Dotu[3], E Raineri[4], S Leidel[2], J Díez[1]
Universitat Pompeu Fabra

10:20-10:35
The Aurora-B dependent NoCut checkpoint prevents damage of anaphase bridges after DNA replication stress
Nuno Amaral, Alexandre Vendrell, Charlotta Funaya, Fatima-Zahra Idrissi, Arun Kumar, Gabriel Neurohr, Neus Colomina, Jordi Torres-Rosell, María-Isabel Geli and Manuel Mendoza
Centre for Genomic Regulation

10:35-10:50
A comparative analysis of Multisubunit Tethering Complexes reveals a new function for Drs2
Irene Pazos, Ana García, Marc Abella, Carla Belmonte, Nere Jiménez and Oriol Gallego
Institut de Recerca Biomèdica de Barcelona

10:50-11:30 Cafè i Pòsters

11:30-11:45
AN INTRINSICALLY DISORDERED REGION OF RPN10 PLAYS A KEY ROLE IN RESTRICTING UBIQUITIN CHAIN ELONGATION IN RPN10 MONOUBIQUITINATION
Pilar Puig-Sàrries, Marie-José Bijlmakers, Alice Zuin, Anne Bichmann, Miquel Pons and Bernat Crosas
IBMB-CSIC

11:45-12:00
Prp45, the yeast ortholog of the human SKIP factor, genetically interacts with the regulation of spliceosome assembly
Mireia Labrador, Josep Vilardell

12:00-12:15
Does the coding sequence determine mRNA levels?
Lorena Espinar, Júlia Domingo, Lucas Carey

12:15-12:30
Human New Cyclins: expression in tumors and novel interactors
Sara Hernández-Ortega, Eva Quandt, Laura Gasa, Mariana PC Ribeiro, Natalia Ricco, Samuel Bru, Javier Jiménez and Josep Clotet
Universitat Internacional de Catalunya

12:30-12:45
NrdR; a single transcription factor behind all dNTP synthesis in the fastidious pathogen Pseudomonas aeruginosa
Lucas Pedraz, Anna Crespo, Eduard Torrents
Institute for Bioengineering of Catalonia

12:45-13:00
Generation of a mouse model by CRISPR/Cas9 system to study retinal function and degeneration

Maria José López-Iniesta[1,2], Roser Gonzàlez-Duarte[1,2,3], Gemma Marfany[1,2,3]
Universitat de Barcelona

13:00-13:15
In search of mitochondrial biomarkers in colon of premotor Parkinson's disease patients
González-Casacuberta I1, Navarro-Otano J2, Juárez DL1, Vilas D2, Garrabou G1, Pont-Sunyer C2, Catalán-García M1, Guitart-Mampel M1, Tobías E1, Cardellach F1, Valldeoriola F2, Tolosa E2α, Morén C1α
IDIBAPS-UB, Hospital Clínic de Barcelona

13:30-15:00 Dinar i Pòsters

Moderador: Gemma Marfany (UB)

15:00-15:15
Sporadic inclusion body myositis patients with mitochondrial DNA deletions show lower mtDNA content and Mitofusin-2 decrease
M Catalán-García[1], G Garrabou[1], C Morén[1], S Emperador[2], M Guitart-Mampel[1], I González-Casacuberta[1], D Juarez[1], A Hernando[1], Jennifer Enrich[1], E Tobias[1], F Cardellach[1], J Montoya[2], JM Grau[1].
IDIBAPS-UB, Hospital Clínic de Barcelona

15:15-15:30
Gastrin stimulates Rgnef through Gα_{13} in DLD-1 colon carcinoma cells
I.Izquierdo, M.Masià, G.Garrido, A.Cordomí, L.Pérez, N.L. Miller, D.D.Schlaepfer, V.Gigoux and A.M. Aragay
IBMB-CSIC

15:30-15:45
Phenotyping mitochondrial lesion in a cell model of Parkinson'sDisease
Juarez-Flores DL1*, Gonzàlez-Casacuberta I1*, Ezquerra M2, Morén C1, Bañó M1, Catalán-Garcia M1, Gaig C2, Guitart-Mampèl M1, Pont-Sunyer C2, Tobías E1, Tolosa E2, Cardellach F1, Fernández-Santiago R2, Garrabou G1.
IDIBAPS-UB, Hospital Clínic de Barcelona

15:45-16:00
Searching for single nucleotide genetic variants (SNVs) associated with disease beyond protein-coding regions: Regulome-seq
Mel·lina Pinsach-Abuin, Sara Pagans, Catarina Allegue, Ivan Garcia-Bassets, Jesus Mates i Bernat del Olmo
Institut d'Investigació Biomèdica de Girona, Universitat de Girona

16:00-16:15
Mitochondrial implication in intrauterine growth restriction and associated cardiovascular remodeling
Guitart-Mampel M*[1,3], Roca-Agujetas V*[1,3], Gonzàlez-Tendero A[2,3], Niñerola S[1,3], Moren C[1,3], Catalán-Garcia M[1,3], González-Casacuberta I[1,3], DL Juárez-Flores[1,3], Tobías E[1,3], Crispi F[2,3], Garrabou G[1,3], Gratacós E[2,3], Cardellach F[1,3].
IDIBAPS-UB, Hospital Clínic de Barcelona

16:15-16:30
Gene silencing by PPRHs: improved design and effect on relevant cancer target genes in different human cell lines
Xenia Villalobos, Carlos J. Ciudad and Véronique Noé
University of Barcelona

16:45-17:20 Conferenciant convidat —*Biologia i Indústria*—

David Resina
CEO, Bioingenium, Parc Científic de Barcelona
Producció de proteïnes recombinants, del laboratori fins la producció industrial

17:30 Pòsters

Dimecres 3 de juny

9:00-9:40 Conferenciant convidat

David Reverter
Universitat Autònoma de Barcelona (UAB)
SUMO: a novel biological pathway that parallels to the Ubiquitin system
Moderadors: Maria Solà (IBMB-CSIC), J. Lourdes Campos (UPC)

9:45-10:00
Crystal structure of human RNase 6 bound to sulphate anions at high resolution: Structural insights into its antimicrobial mechanism of action
Prats-Ejarque G, Arranz-Trullén J; Blanco, JA; Pulido, D; Nogues, MV; Moussaoui M and Boix E.
Universitat Autònoma de Barcelona

10:00-10:15
Prediction of interface hot-spot residues to characterize pathological mutations in protein-protein interactions
Didier Barradas Bautista, Juan Fernández Recio
Barcelona Supercomputing Center

10:15-10:30
Structural insights into the action of a bacterial protease inhibitor
Irene Garcia-Ferrer [1], Pedro Arêde [1], Josué Gómez-Blanco [2], Daniel Luque [2,3], Stephane Duquerroy [4,5], José R. Castón [2], Theodoros Goulas [1,*] and F. Xavier Gomis-Rüth [1,*]
IBMB-CSIC

10:30-10:45
Unravelling Mycoplamsa genitalium gliding motility system from structural studies
Mercè Ratera, Gonzalez-Gonzalez L., Martinelli L., Adell, M., Garcia L., Calisto B., Pinyol J., Querol E., Fita I.
IBMB-CSIC

10:45-11:00
TBA

11:00-11:40 Cafè i Pòsters

11:40-12:20 Conferenciant convidat

Joan-Ramon Daban
Universitat Autònoma de Barcelona (UAB)
Metaphase chromatin plates explain the morphology, dimensions and mechanical properties of condensed chromosomes

12:20-12:35
Structural insights into *Vibrio cholerae* virulence cascade
Simone Pieretti[1,2], Rosa Perez Luque[1,2], Albert Canals[1,2], Eric Krukonis[3], Miquel Coll[1,2]
IBMB-CSIC, **IRB**

12:35-12:50
Identification of the structural and energetic basis of MEK1 pathological mutations: a Molecular Dynamics and Metadynamics study
Chiara Pallara[1] and Juan Fernández-Recio[1]
Barcelona Supercomputing Center

13:00-13:40 Conferenciant convidat

Eduard Batlle
Institut de Recerca Biomèdica de Barcelona (IRB), ICREA
Metastatic Stem Cells and TGF-beta signaling in Colorectal Cancer

13:45 Lliurament del premi *Lluís Cornudella* i comiat
14:00 (Dinar per als conferenciants i IPs)

PÒSTERS

1
Correction of different point mutations of the *dhfr* gene in mammalian cell lines
Anna Solé, Judit Amenós, Carlos J. Ciudad and Véronique Noé

2
Regulation of proteasomal ubiquitin receptor composition by Rpn10 monoubiquitination
Anne Bichmann[1,2], Alice Zuin[1], Marta Isasa[3], Bernat Crosas[1]

3
The deubiquitinating enzyme Ataxin-3 is involved in retinal degeneration in zebrafish
Vasileios Toulis[1], Maria José López-Iniesta[1,3], Silvia Garcia-Monclús[1,4], Mariona Esquerdo[1], Víctor Abad-Morales[1], Alejandro Garanto[1,5], Gemma Marfany[1,2,3]

4
Neuritin-1 gene role in the presence of depressive symptoms and in modulating neurocognitive performance: a general population based study
Claudia Prats[1,2] , Bárbara Arias[1,2] , Jorge Moya[2,3] , Helena Villa[4] , Ignacio Ribes[2,4] , Generós Ortet[2,4] , Lourdes Fañanás[1,2] , Mar Fatjó-Vilas[1,2]

5
***MIR137*, *ZNF804A* and *CACNA1C* genes and the risk for psychotic disorders: a family-based association study.**
Miquel Jorba[1] , Salvador Miret[2,3] , Mª José Muñoz[4] , Victor Peralta[5] , Lourdes Fañanás[1,2] , Mar Fatjó-Vilas[1,2]

6
Novel mode of DNA recognition by HMG proteins

Raquel Sánchez-Giraldo[a], **Francisco J. Acosta-Reyes[a]**, Núria Saperas[a], Cinthia Millán[a], Sonia García[a], Mair Churchill[b] y J. Lourdes Campos[a]

7
Structural analysis of condensed metaphase chromosomes by Synchrotron small-angle X-ray scattering
Andrea Chicano[1], Eva Crosas[2], Christina Kamma-Lorger[2], Juan Carlos Martínez[2], Marc Malfois[2], Agneta Svensson[2], and Joan-Ramon Daban[1]

8
Optimization of protein complex assembly in vitro
Claus-A. Schmitz

9
Searching surface mutations with higher thermostability in the prokaryotic LAT transporter Asc-like. Towards the structure of a LAT transporter
Joana Fort[1,2,3], Paula Santos[1] ,Patricia Pacios[1], Paola Batoccioni[1,2,], Ekaitz Errasti[1], Manuel Palacín[1,2,3]

10
HIV-1 promonocytic and lymphoid cell lines as proper in vitro models of in vivo mitochondrial lesion
Morén C[1], González-Casacuberta I[1],Álvarez-Fernández C[2],Bañó M[1,] ,Catalán M[1],Guitart-Mampel M[1],Tobías E[1],Cardellach F[1],Gatell JM[2],Sánchez-Palomino S[2],Garrabou G[1]

11
Mitochondrial and apoptotic in vitro modelling of differential HIV-1 progression and antiretroviral toxicity
C. Morén[1,2†], M. Bañó[1,2†], I. González-Casacuberta[1,2], M. Catalán-Garcia[1,2], M. Guitart-Mampel[1,2], E. Tobías[1,2], F. Cardellach[1,2], E. Pedrol[3], J. Peraire[4], F. Vidal[4], P. Domingo[5], Ò . Miró[1], J. M. Gatell[6], E. Martínez[6] and G. Garrabou[1,2]

CONTROL OF GENE EXPRESSION BY STRESS-ACTIVATED PROTEIN KINASES (SAPKS)

Carme Solé, Mariona Nadal, Francesc Posas & Eulalia de Nadal

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Exposure of yeast cells to high osmolarity results in the activation of the p38-related Hog1 Stress-Activated Protein Kinase (SAPK), which is required to generate a set of osmoadaptive responses. Adaptation to stress requires the induction of a large number of genes that is highly dependent on the presence of Hog1. The SAPK controls several steps of the transcription process upon stress. At initiation, Hog1 not only directly phosphorylates several transcription factors to alter their activities, but also associates at stress-responsive promoters through such transcription factors. Once at the promoters, Hog1 serves as a platform to recruit general transcription factors, chromatin modifying activities and RNA Pol II. In addition, Hog1 plays a role in elongation. Genome wide analyses have shown that upon stress there is a global redistribution of RNA Pol II associated to Hog1 targeted loci. The presence of Hog1 at chromatin is critical for chromatin reorganization, which facilitates strong gene induction upon stress. In addition, Hog1 associates and controls the induction of a novel set of lncRNAs in response to osmostress. One of the genes expressing a stress-induced lncRNA in antisense orientation is CDC28, the CDK1 kinase that controls the cell cycle in yeast. Induction of the CDC28 lncRNA permits the increase on the levels of Cdc28 allowing cells to re-entry more efficiently cell cycle after stress. Elucidating the control of gene expression by the Hog1 SAPK should help to understand how eukaryotic cells implement a massive and rapid change on their transcriptional capacity in response to adverse conditions.

THE DEAD-BOX HELICASE DHH1 PROMOTES TRANSLATION OF HIGHLY STRUCTURED MRNAS

J Jungfleisch[1], D Nedialkova[2], I Dotu[3], E Raineri[4], S Leidel[2], J Díez[1]

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[2]. RNA Biology Laboratory, Max Planck Institute for Molecular Biomedicine, Münster, Germany

[3]. Hospital del Mar Medical Research Institute, Barcelona, Spain

[4]. Statistical Genomics, Centro Nacional de Analisis Genomica, Barcelona, Spain

Translation control and mRNA decay are central to maintain proper gene expression allowing to respond rapidly to perturbations. The group of Dhh1/DDX6 DEAD-box helicases plays a key role in these processes since its members act at the interface of mRNA translation and decay. They promote translation repression of cytoplasmic mRNAs that are then fed into decay or stored. Intriguingly, we have previously shown that Dhh1/DDX6 activated translation of positive-strand RNA viral genomes. However, the mechanism involved and whether this role is extended to cellular mRNAs is unknown. By using a model system that allows the replication of the Brome mosaic virus in yeast here we show that the ATPase activity of Dhh1 was required for its positive role in translation. Moreover, polysome profile analyses indicated that Dhh1 promotes translation initiation. This role was linked to the concurrent presence of the 5´ and 3´UTRs, two highly structured sequences known to control translation, and of a newly determined stem-loop in the ORF region. Consistent with a direct role of Dhh1 in translation, Dhh1 co-immunoprecipitated with the viral RNA without affecting its stability. Excitingly, genome-wide ribosome profiling analyses in yeast demonstrated that Dhh1 also promotes translation of a specific subset of cellular mRNAs that are enriched in previously described Dhh1-bound mRNAs. These mRNAs present higher base pair probablilities at their ORFs than those translationally-repressed or not translationally affected by Dhh1 and are enriched in mRNAs related to ribogenesis processes. As a consequence modulation of Dhh1 activity will lead to their fast coregulation, as needed for example under stress conditions. In sum, our results uncover a novel role of Dhh1 in the cell that has been hijacked by viruses to control their gene expression and points out at this DEAD-box helicase as a key cross-talk mediator between translation, translational repression and decay.

THE AURORA-B DEPENDENT NOCUT CHECKPOINT PREVENTS DAMAGE OF ANAPHASE BRIDGES AFTER DNA REPLICATION STRESS

Nuno Amaral, Alexandre Vendrell, Charlotta Funaya, Fatima-Zahra Idrissi, Arun Kumar, Gabriel Neurohr, Neus Colomina, Jordi Torres-Rosell, María-Isabel Geli and Manuel Mendoza

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Coordination of cytokinesis with chromosome segregation is essential to maintain genome stability during cell proliferation. In yeast and animal cells, anaphase chromatin bridges induce an abscission delay through the Aurora B-dependent NoCut checkpoint. However, it is not known whether inhibition of abscission prevents damage of these bridges and how they are detected by the checkpoint. We find that chromatin bridges induced by replication stress delay abscission through the NoCut checkpoint. This delay prevents cytokinesis-dependent DNA damage and promotes cellular viability. Similarly, chromatin bridges induced by condensin or topoisomerase II inactivation also trigger an Aurora B-dependent abscission delay. Surprisingly, chromatin bridges from dicentric chromosomes do not affect abscission, indicating that chromatin alone is not sufficient to trigger a NoCut response. The NoCut response after replication stress and in condensin/topoisomerase II mutants induced stabilization of the anaphase spindle during the initial stages of cytokinesis and of the spindle midzone stabilizing protein Ase1/PRC1. Supporting a role for spindle stabilization in NoCut function depolymerization of the anaphase spindle abrogated the abscission delay in topoisomerase II mutant cells. Interestingly, inactivation of the late-anaphase ubiquitin-ligase complex APC-Cdh1 stabilizes the mitotic spindle and introduced an Aurora B-dependent abscission delay in cells with dicentric chromatin bridges. This suggests that APC-Cdh1 substrates are involved in the NoCut response. We propose that chromosomal structural defects, induced by replication stress, decondensation or persistent catenations, trigger NoCut through impairment of APC-Cdh1 activity. This protects its substrates from degradation, stabilizes the mitotic spindle and allows midzone-bound Aurora B to detect chromatin bridges and inhibit abscission.

A COMPARATIVE ANALYSIS OF MULTISUBUNIT TETHERING COMPLEXES REVEALS A NEW FUNCTION FOR DRS2

Irene Pazos, Ana García, Marc Abella, Carla Belmonte, Nere Jiménez and Oriol Gallego.
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Multisubunit Tethering Complexes (MTCs) form a group of 9 protein assemblies essential for vesicle trafficking that are conserved from yeast to human. Each MTC recognizes a specific type of vesicles and tether them to the correct acceptor membrane. Overall, MTCs provide specificity and directionality to vesicle trafficking.

PICT (Protein interaction from Imaging of Complexes after Translocation) is a new technique that allows the study of transient protein-protein interactions directly in living cells. In our laboratory, we use this light microscopy method to characterize MTCs from a functional and structural point of view in the yeast *Saccharomyces cerevisiae*. I will present our work to identify new components that associate transiently with these complexes as well as MTC's cargo specificity profile. We characterized 7 new cargo specific for different MTCs and found evidences for a new function of one of them: the lipid flippase Drs2.

AN INTRINSICALLY DISORDERED REGION OF RPN10 PLAYS A KEY ROLE IN RESTRICTING UBIQUITIN CHAIN ELONGATION IN RPN10 MONOUBIQUITINATION

Pilar Puig-Sàrries, Marie-José Bijlmakers, Alice Zuin, Anne Bichmann, Miquel Pons and Bernat Crosas
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Keywords: Monoubiquitination, polyubiquitin chain, proteasome, E3 ubiquitin ligase, Rpn10, Rsp5, ubiquitin, intrinsically disordered protein, fold-back model

Despite being common mechanism in eukaryotes, the process by which protein monoubiquitination is produced and regulated *in vivo* is not completely understood. We present here the analysis of the process of monoubiquitination of the proteasomal subunit Rpn10, involved in the recruitment of polyubiquitinated substrates. Rpn10 is monoubiquitinated *in vivo* by the Nedd4 enzyme Rsp5, and this modification impairs the interaction of Rpn10 with substrates, having a regulatory effect on proteasome function. Remarkably, a disordered region near the ubiquitin interacting motif of Rpn10 plays a role in the restriction of the polyubiquitin extension activity of Rsp5. Mutations in this disordered region promote ubiquitin chain extension of Rpn10. Thus, our work sheds light on the molecular basis and the functional relevance of a type of monoubiquitination that is driven by the substrate. Moreover, we uncover a putative role for disordered regions in modulating ubiquitin-protein ligation.

PRP45, THE YEAST ORTHOLOG OF THE HUMAN SKIP FACTOR, GENETICALLY INTERACTS WITH THE REGULATION OF SPLICEOSOME ASSEMBLY

Mireia Labrador, Josep Vilardell

DOES THE CODING SEQUENCE DETERMINE MRNA LEVELS?

Lorena Espinar[1], **Júlia Domingo**[1] and Lucas Carey[1].

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Proper control of mRNA levels is critical to ensure biological processes. Deciphering the rules that determine the amount of a gene product requires to fully understand the contribution of all genetic sequences on gene expression. Because in microbial populations the vast majority of the genome is coding, in this study we wanted to understand how the open reading frame (ORF) determines mRNA abundance in yeast. Using a high-throughput sequencing strategy, we built a library with >6000 different ORF sequences from random fragments of the *Saccharomyces cerevisiae* genome. Under the induction of the same promoter the library spans a continuous fourteenth-fold range of expression values. Our analysis reveals a dominant contribution of non-sense codons within the ORF and 3'UTR lengths to phenotypic diversity, where the longer tends to be the aberrant 3'UTR, the less expression the transcript has. We find that DNA-encoded directionality has an impact on mRNA expression. Genomic fragments inserted in the library with the opposite orientation from the found in the genome have lower mRNA levels. Such range of expression values also allowed us to show how optimal codons differ for very overexpressed genes. We finally devised computational models including ORF features that are able to explain >50% of the measured expression variability of our sequence variants. Thus, in steady-state conditions, a large fraction of mRNA variation levels is strongly determined by inherent properties of coding sequences related to mRNA synthesis, stability and protein translation rather than trans or cis-regulatory mechanisms.

HUMAN NEW CYCLINS: EXPRESSION IN TUMORS AND NOVEL INTERACTORS

Sara Hernández-Ortega, Eva Quandt, Laura Gasa, Mariana PC Ribeiro, Natalia Ricco, Samuel Bru, Javier Jiménez and Josep Clotet.
Grup de Noves Ciclines, Departament de Ciències Bàsiques, Universitat Internacional de Catalunya, Josep Trueta s/n, 08195, Sant Cugat del Vallès. jclotet@uic.es

Cell cycle is controlled by CDKs along with their correspondent partner cyclins in each phase of the cycle. Some years ago, our group described in *S. cerevisiae* that some of these cyclins, which seem not to be essential, have special relevance when the environmental conditions are not favorable (Hernández-Ortega *et al.*, JBC, 2013).

Our group is now interested in describing the functions of these novel cyclins that appeared after the Human Genome Project. We are currently investigating their expression pattern in different types of cell lines and tumor tissues. We are also trying to find which are the correspondent CDKs and substrates for each human cyclin by 2-hybrid assays and a proteomic approach.

During the presentation, we will show some preliminary results and the first interactors that we uncovered. Discussion will be welcomed.

NRDR; A SINGLE TRANSCRIPTION FACTOR BEHIND ALL DNTP SYNTHESIS IN THE FASTIDIOUS PATHOGEN *PSEUDOMONAS AERUGINOSA*

Lucas Pedraz, Anna Crespo, Eduard Torrents

Bacterial infections and antimicrobial therapies. Institute for Bioengineering of Catalonia, Barcelona, Spain - lpedraz@ibecbarcelona.eu

Nowadays, the fear of infectious diseases is again increasing. Antibiotic-resistant bacterial strains are appearing worldwide, and so the antibiotic era is coming to an end. There is an urgent need to develop new antimicrobial drugs, new therapies that must be directed to previously known molecular targets.

Ribonucleotide Reductases (RNRs) are essential enzymes for the life of any cell, as they catalyse the reduction of ribonucleotides (NTPs) to their corresponding deoxyribonucleotides (dNTPs), thereby forming the building blocks for DNA synthesis and repair. An antimicrobial drug able to inhibit bacterial Ribonucleotide Reductase activity would be able to completely inhibit bacterial growth.

Behind Ribonucleotide Reductase activity there is a complex regulon; although eukaryotic cells codify only for one RNR enzyme, bacteria can use up to three RNR classes, showing a great metabolic adaptability. *Pseudomonas aeruginosa* is a major human opportunistic pathogen, causing, among other diseases, severe lung chronic infections in cystic fibrosis and COPD (Chronic Obstructive Pulmonary Disease) patients. It codifies for all three RNR classes, and regulates them in a complex manner to adapt to many environments and growth conditions.

The main focus of this work is a transcription factor, called NrdR, which is present in almost all bacterial species, and completely absent in eukaryotic organisms. This factor acts as a central regulator of all RNR enzymes in bacteria, hence being behind all dNTP synthesis. We have studied how NrdR regulates RNR activity in *P. aeruginosa,* being able to this point able to propose a first model of its complex regulon.

GENERATION OF A MOUSE MODEL BY CRISPR/CAS9 SYSTEM TO STUDY RETINAL FUNCTION AND DEGENERATION

Maria José López-Iniesta[1,2], Roser Gonzàlez-Duarte[1,2,3], Gemma Marfany[1,2,3]

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Mutations in over 200 genes are associated to inherited monogenic retinal degenerative diseases (prevalence 1:3000 worldwide), but we are still far from completely understanding their ethiopathology. In our group, we study retinal function at the molecular level to shed light on the molecular bases of retinal degeneration. Therefore, animal models are an essential tool since *in vitro* and cell culture assays cannot provide information of complex interactions among cell types and organs.
Gene modifications in model organisms have been extremely helpful to approach gene function. The most recent and efficient technique to produce targeted genome modifications, or genome editing, is the RNA-guided endonuclease CRISPR/Cas9 system. By using this new approach, we aimed to delete the last exon of *Nr2e3*, a gene that encodes a retinal transcription factor relevant for photoreceptor developmental fate and maintenance. Indeed, mutations in *NR2E3* in human cause severe retinal neurodegenerative diseases.

Nr2e3 is a dual transcription factor that can act both as activator or repressor. It displays several domains, the most significant being a DNA Binding Domain (DBD) and a Ligand Binding Domain (LBD). The deletion at the last coding exon would disrupt part of LBD, required for dimerization and transcriptional repression. Thus, although there is a natural mouse mutant, rd7, which generates a null allele by early Nr2e3truncation, this new mutant would greatly help us to further dissociate the different functional roles of this transcription factor.

After our design and in collaboration with the Mouse Mutant Core Facility in the IRB (Barcelona), mouse zygotes were microinjected with several RNA-guides, the Cas9 D10A mRNA (a modified version that acts as a nickase) and a double stranded oligonucleotide designed to target and connect the ends of the deletion. The genotyping process of the genome-edited progeny, assays for checking possible off-target events and mating strategies will be presented.

IN SEARCH OF MITOCHONDRIAL BIOMARKERS IN COLON OF PREMOTOR PARKINSON'S DISEASE PATIENTS

González-Casacuberta I1, Navarro-Otano J2, Juárez DL1, Vilas D2, Garrabou G1, Pont-Sunyer C2, Catalán-García M1, Guitart-Mampel M1, Tobías E1, Cardellach F1, Valldeoriola F2, Tolosa E2α, Morén C1α

1 Muscle Research and Mitochondrial Function Laboratory, Cellex-IDIBAPS, Faculty of Medicine-University of Barcelona, Internal Medicine Department-Hospital Clínic of Barcelona-CIBERER U722, Barcelona, Spain; 2 Neurology Service, Hospital Clinic de Barcelona, IDIBAPS, CIBERNED, Barcelona, Spain; α Equally contribution.

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Introduction

There is wide evidence of mitochondrial involvement in neurological diseases, especially in Parkinson's disease (PD). First associations between mitochondrial dysfunction and PD were Complex I (CI) impairment and the consequent increase in oxidative stress in postmortem *substantia nigra* of PD patients. These deficiencies have also been described in peripheral tissues.

The majority of patients with REM Behaviour Disorder (RBD) are in prodromal stages of PD neurodegeneration. Thus, the search of a mitochondrial biomarker in peripheral tissue, such as colon, of RBD patients would allow to find a less invasive prognosis of PD before the onset of motor symptoms.

Methods

Homogenization of sigmoid colon biopsies was performed in 13 patients presenting motor PD, 7 RBD patients and 9 controls.

We assessed mitochondrial content through citrate synthase activity and the respiratory chain function by CI measurement by spectrophotometry. The oxidative stress was determined by lipid peroxidation through malondialdehyde and 4-hydroxyalkenals quantification by spectrophotometric measurement.

Results

A slight decrease in mitochondrial content was found in PD and RBD patients compared to controls (175.08±16.6 vs 159.45±19.12 vs 196.58±17.24, p=NS). CI activity tended to decrease in PD and RBD patients compared to controls (54.97±15.76 vs 45.69±18.15 vs 59.51±12.52, p=NS). Oxidative stress was slightly increased in PD patients compared to RBD and controls (33.43±3.79 vs 27.46±3.04 vs 31.71±3.9, p=NS).

Conclusions

These findings may suggest that mitochondrial impairment plays a role in the ethiopathogenesis of PD because it is present before motor symptomatology begins and suggest the potential use of mitochondrial prognostic biomarkers in the pre-motor stage of PD.

A slight increase in oxidative stress was only found in PD patients suggesting that this mechanism is present in advanced motor stages of the disease as a consequence of CI dysfunction. Nevertheless, further research on other putative mitochondrial targets is needed to explore other potential altered prognostic factors.

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SPORADIC INCLUSION BODY MYOSITIS PATIENTS WITH MITOCHONDRIAL DNA DELETIONS SHOW LOWER MTDNA CONTENT AND MITOFUSIN-2 DECREASE

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Introduction

Sporadic inclusion body myositis(sIBM) is the most common inflammatory myopathy in elderly. Recent studies have described the presence of mtDNA deletions in around 70% of those patients. We aimed to assess if sIBM patients with mtDNA deletions present increased mitochondrial abnormalities, such as mtDNA depletion, recently associated to reduced expression of Mitofusin-2(MFN2), a protein involved in a key process for mitochondrial survival: mitochondrial fusion and renewal.

Methods

Leftover materials from 14 sIBM patient muscle biopsies were immediately frozen at -80ºC until analysis. In addition, 17 muscle biopsies from free-of-muscle disease age and gender-paired controls were parallelally included. Total DNA was isolated by the fenol-chloroform technique. Mitochondrial DNA deletions were assessed by Long-PCR. Mitochondrial DNA amount was assessed by quantitative real time PCR, and values were expressed as the ratio between mitochondrial 12SrRNA gene respect to the nuclear RnaseP gene amount. Mitofusin-2 levels were quantified by Western-blot, and α-tubulin was used as a loading control. Values were expressed as the ratio between MFN2/α-tubulin expression.

Results

Eight out of 14(57%) of the sIBM patients presented multiple or single mtDNA deletions and 6 of them were clearly negative. MtDNA amount was slightly decreased in sIBM patients with mtDNA deletions compared to sIBM without deletions. These differences became statistically significant when comparing sIBM patients with deletions with respect to the control group. MFN2 levels resembled the pattern of mtDNA amount showing the statistically significant lowest content in sIBM patients with mtDNA deletions and the highest level in controls, being sIBM patients without mtDNA deletions those presenting an intermediate value of expression.

Conclusion

Sporadic inclusion body myositis present three pathological features: inflammation, degeneration and mitochondrial abnormalities. This study deepens in the understanding of mitochondrial processes, showing how mtDNA deletions could affect other mitochondrial parameters in those patients leading to a deregulation of mitochondrial homeostasis.

GASTRIN STIMULATES RGNEF THROUGH GA_{13} IN DLD-1 COLON CARCINOMA CELLS

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The **R**ho **g**uanine **n**ucleotide **e**xchange **f**actor Rgnef (also known as ArhGEF28 or p190RhoGEF) promotes colon carcinoma cell motility and tumor progression via interaction with focal adhesion kinase (FAK). Mechanisms of Rgnef activation downstream of integrin or G protein-coupled receptors remain undefined. In the absence of a recognized G protein signaling homology domain in Rgnef, no proximal linkage to G proteins was known. Utilizing multiple methods, we have identified Rgnef as a new effector for Ga_{13} downstream of gastrin and the type 2 cholecystokinin receptor. In DLD-1 colon carcinoma cells depleted of Ga_{13}, gastrin-induced FAK Tyr(P)-397 and paxillin Tyr(P)-31 phosphorylation were reduced. Rgnef increased RhoA GTP binding and promoter activity in combination with active Ga_{13}. Rgnef co-immunoprecipitated with activated Ga_{13}Q226L but not Ga_{12}Q229L. The Rgnef C-terminal (CT, 1279–1582) region was sufficient for co-immunoprecipitation, and Rgnef-CT exogenous expression prevented Ga_{13}-stimulated SRE activity. A domain at the C terminus of the protein close to the FAK binding domain is necessary to bind to Ga_{13}. Point mutations of Rgnef-CT residues disrupt association with active Ga_{13} but not Ga_q. These results show that Rgnef functions as an effector of Ga_{13} signaling and that this linkage may mediate FAK activation in DLD-1 colon carcinoma cells.

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PHENOTYPING MITOCHONDRIAL LESION IN A CELL MODEL OF PARKINSON'SDISEASE

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Introduction:

Parkinson's Disease (PD) is a highly prevalent, neurodegenerative disorder that affects primarily population over 60 years old, but there are also young onset cases, where the symptoms appear at earlier ages, and are associated to worst outcome and a clear hereditary pattern.

It is widely demonstrated that mitochondrial Complex I (CI) dysfunction induces symptoms of Parkinsonism, We aimed to validate a cell model of PD to establish disease aetiology, biomarkers and therapeutic strategies by first characterizing CI dysfunction in different subsets of patients.

Methods:

Fibroblasts from 5 groups of patients were obtained by skin biopsy and analysed; i: controls, ii: PARKIN mutation carriers, iii: LRRK2 mutation carriers, iv: NMC (non manifesting carriers of LRRK2 mutation) and v: IPD (Idiopathic PD). We assessed CI respiratory chain oxidative and enzymatic function by measurement through polarography and spectrophotometry by using e cellular substrates for CI and comparing CI activity with respect to controls.

Results:

CI-mediated oxygen consumption tended to decrease in all PD groups (PARKIN: -37.45%±16.00; LRRK2 -37.30%±15.43; NMC -26.12%±16.59 and IDP -33.80%±16.31). CI enzymatic activity showed the same pattern, decreasing in PD patients compared to controls (PARKIN: -37.32±12.58; NMC -31.53±11.89; IDP -41.83±17.27) although a slight increase at the basal state was found in LRRK2 (4.75±47.98). When fibroblasts were exposed to a galactose medium designed to force mitochondrial metabolism, patients trended to exacerbate mitochondrial function with respect to controls (mean increase 108.81±49.18 for oxidative and 109.38±50.65 for enzymatic CI function).

Conclusions:

We have demonstrated a decrease in CI oxidative and enzymatic activity of fibroblasts in the PD groups, suggesting a basal mitochondrial dysfunction, which becomes even more evident when stressing cells in a galactose medium. These data validate the presented cell model of PD to further investigate bioenergetic differences among groups and establish specific pathways and therapeutic targets to prevent and reverse PD.

SEARCHING FOR SINGLE NUCLEOTIDE GENETIC VARIANTS (SNVS) ASSOCIATED WITH DISEASE BEYOND PROTEIN-CODING REGIONS: REGULOME-SEQ

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Single nucleotide variants (SNVs) are DNA sequence variations in the human genome. SNVs are important because they are associated with individual -and population-specific susceptibility to disease. Until now, however, their study has been largely limited to the 1-2% human genome with protein-coding potential (exonic regions). In contrast, recent genome-wide association studies (GWAS) have shown that most disease-associated SNVs lie within nonexonic regions, especially in *cis*-regulatory regions. The study of SNVs at nonexonic regions requires massive sequencing, which is rather expensive and laborious, not feasible for most laboratories, and unrealistic for the profiling of large human populations.

We propose to develop a highly cost-effective approach feasible for large scale SNV studies, referred to as regulome-sequencing (Regulome-seq), which is based on a step previous to sequencing of selective capture of *cis*-regulatory regions potentially linked to a disease-associated gene. Although still abundant, *cis*-regulatory regions represent a limited fraction of the human genome, which requires less amount of sequencing to focus on those genomic regulatory regions more likely to be associated with disease. To identify *cis*-regulatory regions, we combine information of topological organization in the human genome, chromatin accessibility, histone marks, and binding of transcriptional regulators.

As a proof-of-principle of the applicability of Regulome-seq to search for disease-associated SNVs in large human populations, we are testing this approach in our biobank of Brugada Syndrome (BrS) DNA samples. BrS is an electrical disease in the heart with high susceptibility to ventricular arrhythmia and sudden cardiac death. SNVs in exonic regions of BrS-associated genes account for 25-30% of BrS cases. We identified candidate *cis*-regulatory regions of BrS-associated genes to profile disease-associated SNVs in these regions. For causality testing, we propose to engineer these variants by CRISPR-Cas9 in human induced pluripotent stem cell (iPSC)-derived cardiomyocytes and establish their effects in gene expression of BrS-associated genes and electrophysiology.

MITOCHONDRIAL IMPLICATION IN INTRAUTERINE GROWTH RESTRICTION AND ASSOCIATED CARDIOVASCULAR REMODELING

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Introduction: Intrauterine growth restriction (IUGR) is an adverse obstetric manifestation with clinical consequences in adulthood. Newborns with IUGR develop idiopathic cardiovascular remodeling. Transcriptome analysis of hearts in IUGR-offspring from a rabbit model showed altered expression of complex-I (CI) function and oxygen-consumption of mitochondrial respiratory chain (MRC). We aimed to determine the implication of mitochondrial function in cardiovascular remodeling of IUGR-newborns from human pregnancies and animal model.

Methods: Peripheral and cord blood mononuclear cells (PBMC and CBMC) were isolated from 39 pregnant women and their newborns (19 IUGR vs.20 controls). In parallel, mitochondria from placenta were isolated. Oxygen-consumption was measured by polarography using endogen cellular substrates (Cellox) and/or substrates for CI (GMox). In the rabbit model: 25 hearts of the offspring (15 IUGR vs.10 controls) and 31 placentas (17 IUGR vs.14 controls) were analyzed. Stimulated CI-oxygen consumption (GMox) was measured by polarography, enzymatic activity of CI, CII, CIV, CII+III, CI+III and oxidative stress by spectrophotometry and ATP levels by flourometry.

Results: In human pregnancies, we found a decrease of maternal and fetal Cellox and also GMox in IUGR-PBMC and CBMC (all p=NS) as well as a significant materno-fetal correlation of GMox ($p<0.05$). We also observed a decrease of GMox in placental mitochondria (p=NS). In the animal model, GMox showed a slight decrease in IUGR-hearts and placentas (both p=NS). Enzymatic activities of CI, CIV, CI+III of MRC decreased in IUGR-hearts and placentas (all p=NS), being significant the enzymatic activities of CII and CII+III (both $p<0.05$). Finally, oxidative stress and ATP levels were reduced in IUGR-hearts ($p<0.001$ and p=NS, respectively), but increased in IUGR-placentas (p=NS and $p<0.01$, respectively).
Conclusions: Experimental results in both animal model and human pregnancies indicate that mitochondrial dysfunction could be related to the development of IUGR and associated cardiovascular remodeling.

GENE SILENCING BY PPRHS: IMPROVED DESING AND EFFECT ON RELEVANT CANCER TARGET GENES IN DIFFERENT HUMAN CELL LINES

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The modulation of gene expression by nucleic acids has become a widely used tool in biomedical research for target validation and to establish new therapeutic approaches. Recently, we developed a DNA-based silencing molecule named polypurine reverse Hoogsteen hairpins (PPRHs). In this work, we explored different characteristics of PPRHs to improve their usage as a tool for gene silencing: we studied the role of PPRH length in the range from 20 to 30 nucleotides finding out that the larger the PPRH the more effective they are in terms of cell viability. To restrict possible off-target effects, we tested wild-type PPRHs, that were capable to bind to their target sequence with more affinity and to produce a higher effect on cell viability, compared to the regular PPRHs. Moreover, we proved that PPRHs show higher affinity of binding and efficacy on cell viability compared to TFOs. Finally, we developed a brand new molecule called Wedge-PPRH and proved its efficacy in prostate and breast cancer cell lines. After evaluating these characteristics, we studied the ability of PPRHs to silence a variety of relevant cancer related genes in several human cell lines. We designed PPRHs against the following genes: BCL2, TOP1, MTOR, MDM2 and MYC. Although all PPRHs were effective, the most remarkable results were obtained with those against BCL2 and MTOR in decreasing cell survival, mRNA levels and increasing apoptosis in prostate, colon and pancreatic cancer cells. In the case of TOP1, MDM2 and MYC, their corresponding PPRHs produced a strong effect in decreasing cell viability and mRNA levels, and increasing apoptosis in breast cancer cells. Thus, we confirm that the PPRH technology is broadly useful to silence the expression of cancer related genes.

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Conferenciant convidat —*Biologia i Indústria*—

David Resina
CEO, Bioingenium, Parc Científic de Barcelona
PRODUCCIÓ DE PROTEÏNES RECOMBINANTS, DEL LABORATORI FINS LA PRODUCCIÓ INDUSTRIAL

Conferenciant convidat

David Reverter
Universitat Autònoma de Barcelona (UAB)
SUMO: A NOVEL BIOLOGICAL PATHWAY THAT PARALLELS TO THE UBIQUITIN SYSTEM

ESTRUCTURA CRISTAL·LINA A ALTA RESOLUCIÓ DE LA RNASA 6 HUMANA UNIDA A ANIONS SULFAT: UNA VISIÓ ESTRUCTURAL EN EL MECANISME D'ACCIÓ ANTIMICROBIANA

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La superfamília de la ribonucleasa pancreàtica bovina (RNasa A) és una família específica de vertebrats que inclou vuit membres funcionals en humans. A part de l'activitat catalítica contra el RNA, s'han descrit en la família altres propietats biològiques implicades en la immunitat[1]. La RNasa 6 és una proteïna catiònica de secreció que s'expressa en macròfags en resposta a una infecció bacteriana[2]. El nostre grup ha resolt per primera vegada l'estructura cristal·lina de la RNasa 6 humana a 1,72 Å. S'hi observa conservació del plegament globular que presenten les altres estructures conegudes de la família. D'altra banda, s'han trobat tres sulfats funcionals units a la RNasa 6, que correspondrien al centre actiu i a dos possibles llocs secundaris d'unió a fosfat.

A més a més, l'anàlisi de l'estructura suggereix una regió potencial d'agregació a la zona C-terminal, la qual podria explicar la seva alta activitat d'aglutinació bacteriana[3]. Finalment, les simulacions *in silico* d'unió al lligand amb diversos dinucleòtids mostren conservació dels principals centres d'unió de purina i pirimidina. Malgrat això, la RNasa 6 presenta una afinitat d'unió amb els lligands més baixa en comparació amb la RNasa A, com ja s'havia observat en estudis cinètics previs realitzats al grup. A més a més, s'estan realitzant estudis funcionals per mutagènesi dirigida per tal d'elucidar el paper de residus específics en l'activitat antipatògena i enzimàtica. Aquests estudis estructurals facilitaran la comprensió del mecanisme d'acció de la RNasa 6, podent ser d'utilitat per al disseny de nous fàrmacs antimicrobians.

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PREDICTION OF INTERFACE HOT-SPOT RESIDUES TO CHARACTERIZE PATHOLOGICAL MUTATIONS IN PROTEIN-PROTEIN INTERACTIONS

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New generation sequencing projects have generated a vast amount of information about changes in a gene sequence and how they can affect individuals. Gene variants, like non-synonymous Single Nucleotide Polymorphisms (nsSNPs), are responsible for diversity among populations. Unfortunately, many of these nsSNPs are also involved in the development of pathological situations. Many of these nsSNPs can affect protein-protein interactions (PPIs) that may be involved in essential cellular processes, such signaling, proliferation, or gene regulation. In order to understand the effect of these nsSNPs at molecular level, it is essential to know the 3D structure of the protein-protein complexes affected by such disease-associated mutations[1], [2]. However, in spite of their importance, there is no available 3D structure for the vast majority of known PPIs[3]. Computational methods, such as protein docking, can complement existing experimental efforts and help building the human structural interactome[4]. The main problem for interactomics application is that accurate prediction of protein-protein structure by docking is still very challenging for many cases. Fortunately, the identification of interface residues, based on sequence conservation or on physico-chemical properties, is more accurate and can be applied at more large scale. When characterizing PPI interfaces, it would be important to identify hot-spot residues, which are those that contribute significantly to the binding energy[5]. We previously developed a method to predict interface residues from docking simulations, called pyDockNIP[6]. The method is able to identify interface hot-spots with high precision, and has the clear advantage of not needing prior information of the complex structure. Here we have developed and validated a variation of this method that can be applied to identify pathological mutations that are involved in PPIs. Our method finds 40% of the known interface nsSNPs with 75% precision. We predict 34% additional nsSNPs that could be involved in interactions.

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STRUCTURAL INSIGHTS INTO THE ACTION OF A BACTERIAL PROTEASE INHIBITOR

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Proteases are essential enzymes in every organism, being involved in many fundamental biological processes from nutrition, tissue remodeling to virulence. Therefore, their activity must be tightly regulated to avoid non specific proteolysis or to defend cells against proteolytic attacks, a function that may be accomplished by protease inhibitors. Contrary to the *metazoans* genomes where protease inhibitors represent 1% of the genes, their appearance in unicellular and specially prokaryotic organisms is much less common, and only few of them have been described.

In our study we describe the mechanism of action of a multidomain, 180kDa, alpha-2-macroglobulin-like protease inhibitor encoded in *Escherichia coli* genome, by applying biochemical and structural techniques, specially X-ray crystallography and cryo-electron microscopy. We demonstrate that the protein is a target for proteases of diverse catalytic mechanism and specificity, that cut in an unstructured bait region. This triggers a big conformational rearrangement in the molecule from a native to an induced form. However, the inhibitor remains monomeric, contrarily to the tetrameric state of some mammalian a2Ms, and the entrapment of the protease is necessarily accomplished by covalent binding through a conserved and highly reactive thiolester bond to a surface lysine of the protease. As a consequence, the protease becomes sterically hindered to reach globular substrates of high molecular weight, so its proteolytic activity is inhibited. Taking into account the periplasmatic localization of the inhibitor, we hypothesize that it is acting as an *E. coli* defense mechanism against invading proteases that may damage cell wall components.

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UNRAVELLING *MYCOPLAMSA GENITALIUM* GLIDING MOTILITY SYSTEM FROM STRUCTURAL STUDIES

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Mycoplasma genitalium is a motile, self-replicating human pathogen. Because *M. genitalium* is a prevalent and emerging sexually transmitted infection, unravelling the molecular basis of its virulence factors is fundamental. *M. genitalium* presents an asymmetrical flask-shape due to a characteristic protrusion formed by the cytoskeleton: the terminal organelle (TO). This complex structure is considered to be the scaffold for mycoplasmas adherence to host cells, mediates cell division and configures a new type of gliding motility.

As this novel prokaryotic motility mechanism is the key factor for the pathogenic infection we focused a top-down structural study on the TO molecular supra-structure, from cell to atomic resolution. To date, up to twelve distinct multi-domain proteins have been localized and is known that formed the TO. We developed several structural studies on TO recombinant proteins and in vivo function experiments with gene-deleted mutants which revealed some main molecular factors involved in the interaction between these proteins and their functions on the cell. We present some interactions found to be major determinants for gliding motility. Among the proteins in the TO, two major adhesins (MgpB-P140 and MgpC-P110), mainly found at the membrane of the TO, formed a nap-like structure outside of the membrane responsible of the host immune response. Due to the antigenic variation of these adhesins *M. genitalium* evade the host immune system causing an inefficient treatment and persistent infection. By three different approaches we want to decipher the molecular supra-structure of this adhesin-complex and zoom in to its atomic molecular resolution. So cryo-tomography of the whole cell and single particle electron microscopy analysis of the endogenous purified adhesin-complex converge in a first low-resolution model of the cell-endogenous and purified nap-like supra-structure. We also present the ongoing X-ray crystal studies studies of the two recombinant adhesin proteins, MgpB and MgpC.

METAPHASE CHROMATIN PLATES EXPLAIN THE MORPHOLOGY, DIMENSIONS AND MECHANICAL PROPERTIES OF CONDENSED CHROMOSOMES

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Previous studies showed that during mitosis chromatin filaments are folded into multilayer plates (1). These structures can be self-assembled from chromatin fragments obtained by micrococcal nuclease digestion of metaphase chromosomes (2). Chromosomes of different animal and plant species show great differences in size (which are dependent on the amount of DNA that they contain), but in all cases chromosomes are elongated cylinders that have relatively similar shape proportions (the length to diameter ratio is approximately 13). It is possible to explain this morphology by considering that chromosomes are self-organizing supramolecular structures formed by stacked layers of planar chromatin having different nucleosome-nucleosome interaction energies in different regions (3). The nucleosomes in the periphery of the chromosome are less stabilized by the attractive interactions with other nucleosomes and this generates a surface potential that destabilizes the structure. Chromosomes are smooth cylinders because this morphology has a lower surface energy than structures having irregular surfaces. The symmetry breaking produced by the different values of the surface energies in the telomeres and in the lateral surface explains the elongated structure of the chromosomes. The results obtained by other authors in nanomechanical studies of chromatin and chromosome stretching have been used to test the proposed supramolecular structure. It is demonstrated quantitatively that internucleosome interactions between chromatin layers can justify the work required for elastic chromosome stretching. Chromosomes can be considered as hydrogels with a lamellar liquid crystal organization. The good mechanical properties of this structure may be useful for the maintenance of chromosome integrity during mitosis. Furthermore this chromatin organization avoids random entanglement of the extremely long genomic DNA molecules in chromosomes.

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STRUCTURAL INSIGHTS INTO *VIBRIO CHOLERAE* VIRULENCE CASCADE

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Membrane-localized ToxR, in association with another membrane protein named TcpP, activates the *toxT* promoter in *Vibrio cholerae*, initiating a regulatory cascade that culminates in the secretion of the cholera toxin and the expression of a pilus, coregulated by the toxin [1]. Both ToxR and TcpP work as a two-component regulatory system merged in single proteins: they receive an external signal through its periplasmic C-terminal domain and bind to the *toxT* promoter by their cytoplasmic N-terminal domains. We are structurally characterizing the system by analyzing ToxR/TcpP-DNA complexes, since two molecules of each transcription factor are supposed to bind the promoter to recruit the RNA polymerase and activate transcription. We have already solved the structure of two complexes of the ToxR DNA-binding domain with 20-bp and 40-bp oligonucleotides using X-ray crystallography.

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IDENTIFICATION OF THE STRUCTURAL AND ENERGETIC BASIS OF MEK1 PATHOLOGICAL MUTATIONS: A MOLECULAR DYNAMICS AND METADYNAMICS STUDY

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Protein kinases are key regulators of eukaryotic living cells, since they are involved in crucial biochemical functions and signaling networks. These enzymes share a common fold and, in response to specific cellular signals, can switch between distinctive inactive and active states through large conformational changes mainly involving the activation loop (A-loop) and the Asp-Phe-Gly (DFG) motif.[1]

One relevant example is the MAPK/ERK cascade, which is involved in important cell processes like gene expression, cell differentiation and apoptosis. Dysregulation of MEK1/2, a key component of MAPK/ERK cascade, is known to cause different serious pathologies, involving several cancer types (melanoma, lung and ovarian cancer) or different congenital anomaly disorders, such as the Cardio-Facio-Cutaneous (CFC) syndrome.[2-3] Unfortunately, despite many MEK1 crystal structures have been already solved the detailed mechanism of MEK1 activation and the functional impact of its pathological mutations remain still unclear. Here we present a systematic study focused on the intrinsic propensity for active-inactive transition in MEK1 WT and in two CFC syndrome-related mutants (Y130C and Q56P), using both long-time scale MD on specific biologically relevant states and extensive *parallel tempering* metadynamics (*PTMetaD)*.[4] The analysis of the MD simulations confirm the regulatory role of A-Helix and A-Loop in the inactive-active transition of MEK1, as previously described and discloses intriguing clues on the specific effects of each mutation, Y130C mutant simulations suggest an increase of the MEK1 basal activity while Q56P shows an over-activation of the phosphorilated state. In addition, the reconstruction of the free energy surface using PT-metaD clarifies the intrinsic activity of the WT and mutants in term of the stability of the DFG-motif conformation.

We hope that these findings could contribute to elucidate *some aspect of the MEK1 activation in physiological and pathological* condition and finally promote the design of new therapies for cancer and CFC-syndrome.

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METASTATIC STEM CELLS AND TGF-BETA SIGNALING IN COLORECTAL CANCER

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About 40-50% of Colorectal Cancer (CRC) patients with locally advanced disease show resistance to therapy and develop recurrent cancer over the course of their treatment. Current CRC staging based on histopathology and imaging has a limited ability to predict prognosis. A major advance has been the elaboration of molecular classifications based on global gene expression profiles, which have defined CRC subtypes displaying resistance to therapy and poor prognosis. We have recently evaluated these molecular classification systems and discovered that their predictive power arises from genes expressed by stromal cells rather than by epithelial tumor cells. Our functional dissection of CRC progression shows that metastasis relies on a tumor cell non-autonomous program driven by TGF-beta in the microenvironment. Virtually all poor prognosis CRC subtypes upregulate this stromal gene program, which confers a survival advantage to metastatic stem cells during organ colonization. Here I will discuss our latest data about the dichotomy of TGF-beta signaling in epithelial versus stromal cells during CRC progression and the use of patient derived tumor organoids and mouse models to test the efficacy of anti-TGF-beta therapies for CRC treatment.

1

CORRECTION OF DIFFERENT POINT MUTATIONS OF THE *DHFR* GENE IN MAMMALIAN CELL LINES

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Repair of single-point mutations in a gene within its endogenous locus has been the focus of active research. However, this goal has not been completely fulfilled and still more methods are needed to improve gene correction. In this direction, an alternative method could be the usage of Polypurine reverse Hoogsteen hairpins (PPRHs). PPRHs are formed by two antiparallel homopurine strands, linked by a five-thymidine loop and constitute a new gene therapy tool. One of the homopurine strands binds to the polypyrimidine target sequence by Watson–Crick bonds with antiparallel orientation forming a triplex. In this study we designed different repair-PPRHs, all containing a Hoogsteen hairpin core, extending one of its strands with a tail of 25nt complementary to the point mutation region of the target, except for the position of the mutated nucleotide to be corrected. The rational of this approach is that the hairpin core of the repair-PPRH binds to the target-DNA and that the extended tail would recombine with the mutated region, thus fixing the mutation. As a model, we used the dihydrofolate reductase (*dhfr*) gene to repair different types of point mutations at a cellular level, in various CHO cell lines. DA5 cells contain a deletion of a guanine in exon 6. The same position is affected by a thymine substitution in DF42 cell line. DA7 cells contain a substitution of a thymidine in exon 3. Finally, DU4.5 cell line bears a substitution in exon 6. All these mutations cause stop codons in the *dhfr* gene (nonsense mutations). In all cases, the selection for *dhfr* positive cells was performed using a culture medium lacking glycine, thymidine, and hypoxanthine (-GHT). The positive colonies were analyzed by DNA-sequencing, for mRNA expression by RT-qPCR, protein content by Western analysis, and DHFR enzymatic activity using the [6-^{3}H]-deoxyuridine assay. Repaired colonies were obtained with each approach. These results suggest that repair-PPRHs could provide the basis for a new therapeutic alternative to repair single-point mutation diseases.

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2

REGULATION OF PROTEASOMAL UBIQUITIN RECEPTOR COMPOSITION BY RPN10 MONOUBIQUITINATION

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Proteolysis mediated by the Ubiquitin-proteasome System (UPS) controls the half-life of numerous regulatory proteins and is a complex process involved in multiple aspects of cell physiology in eukaryotes. Polyubiquitinated proteins are targeted to and degraded in the proteasome, a central ATP-dependent eukaryotic protease. Polyubiquitin receptors, including Rpn10 and Dsk2, selectively recognize and bind polyubiquitinated substrates and are involved in the recruitment of substrates to the proteasome. Rpn10, an intrinsic proteasomal receptor, is found in the cell in proteasome-bound and unbound pools and interacts with the shuttle factor Dsk2. The interaction between extraproteasomal Rpn10 and Dsk2 has been proposed to regulate the amount of Dsk2 that gains access to the proteasome. However, how the recruitment of substrate receptors is regulated remains largely unknown.

Monoubiquitination of Rpn10 has emerged as a conserved mechanism with strong impact on Rpn10 function. We obtained *in vitro* data that show that the Rsp5-dependent monoubiquitination of proteasomal Rpn10 drives the dissociation of Rpn10 from the proteasome promoting an extraproteasomal pool of monoubiquitinated Rpn10 (mUb-Rpn10). Moreover, our data further suggest that extraproteasomal mUb-Rpn10 is not able to interact with Dsk2 due to its inactive UIM domain, allowing Dsk2 to deliver its substrates to the proteasome. Therefore, monoubiquitination of Rpn10 could control the recruitment of Rpn10 and Dsk2 that could lead to a change of substrate receptor composition of the proteasome. In fact, we were able to purify Dsk2 rich proteasomes from a rpn10Δ strain previously transformed with a plasmid expressing a Rpn10-Ub chimera (Ubiquitin attached to the C terminus of Rpn10) and Dsk2 poor proteasomes from a WT strain. Our results reveal a new mechanism of regulation of proteasome function that sheds light on recruitment and shuttling of substrate receptors.

3

THE DEUBIQUITINATING ENZYME ATAXIN-3 IS INVOLVED IN RETINAL DEGENERATION IN ZEBRAFISH

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The gene *Ataxin 3* (*ATXN3*) is involved in the Machado–Joseph Disease (MJD), also known as Spinocerebellar Ataxia Type 3 (SCA3), a late onset autosomal dominant neurodegenerative disorder that belongs to the group of polyglutamine (polyQ) diseases. The ATXN3 protein displays a josephin catalytic domain characteristic of the MJD subfamily of deubiquitinating enzymes (DUBs). Much is known on the neuronal toxicity of the polyQ aggregates but the physiological role of ATXN3 is not completely understood. Given that post-translational modifications, such as SUMOylation and ubiquitination seem to play a crucial role in photoreceptor development and the determination of photoreceptor cell fate, we aimed to explore the function of ATXN3 in retinal development by performing quantitative RT-PCR, *in situ* hybridization and immunofluorescence detections in mouse and zebrafish retinas. The functional analysis was approached by knocking-down *Atxn3* expression by morpholino microinjection in zebrafish embryos, and we also studied the phenotypic rescue by co-microinjection of mRNA from the human gene.

The expression pattern and mRNA levels of *Atxn3* was studied and compared in both mouse and zebrafish. *Ataxin 3* was also knocked down in zebrafish embryos in order to analyze the potentially morphant phenotypes, which were rescued with human ATXN3Q22WT, ATXN3Q22C14A (catalytically inactive form) and ATXN3Q80 (SCA3 form) mRNAs.

CONCLUSIONS: A comparative *Atxn3* expression map in P60 mouse and 7 days zebrafish retinal cryosections has been drawn, showing evolutionary conservation in mRNA localization. A knockdown model for *Ataxin-3* has been generated in zebrafish, and the morphants showed severe ocular morphological defects, with a defective formation of the retinal structures: namely, no lamination, no observable plexiform layers nor differentiated photoreceptors. Moreover, evolutionary conservation was further reinforced by the phenotypic rescue observed when microinjecting three different human *Ataxin 3* mRNAs. These results support the involvement of ATXN3 in the formation and differentiation of the vertebrate retina.

4

NEURITIN-1 GENE ROLE IN THE PRESENCE OF DEPRESSIVE SYMPTOMS AND IN MODULATING NEUROCOGNITIVE PERFORMANCE: A GENERAL POPULATION BASED STUDY

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Background: Depressive symptoms are frequent in the general population, varying between 2.1% and 7.6% (Regier et al 1988). It is proposed that impaired mechanisms of neuroplasticity could underlie these symptoms. Neuritin-1 gene (*NRN1*), also called candidate plasticity gene 15, operates as an intercellular signal between neighbouring neurons (Naeve et al 1997). Animal models have shown that, Neuritin knockdown results in depressive behaviors and antidepressant treatment changes *NRN1* expression levels (Son et al 2012). Our study seeks to investigate in a general population based sample whether the *NRN1* gene contributes to: i) the presence of depressive symptoms, ii) variability in cognitive performance.

Methods: A sample of 439 individuals (mean age (sd): 22.09(3.5); 44.2% male) from the general population filled in a self-reported psychological symptoms questionnaire (The Brief Symptom Inventory (BSI, Ruipérez et al 2001)). Executive function was explored by means of: i) phonemic and semantic fluency, ii) letter-number subscale of Wechsler Adult Intelligence Scale. Eleven SNPs in NRN1 (SNP1-SNP11:rs2208870-rs12333117-rs582186-rs645649-rs582262-rs3763180-rs10484320-rs4960155-rs9379002-rs9405890-rs1475157) were genotyped (TaqMan assays). Association analyses were conducted with Plink 1.07. Age and sex, were included as covariates when appropriate.

Results: GG homozygous of rs1475157 showed higher scores on BSI depressive symptoms than A allele carriers (β=0.62, p=0.00036). The same genotype was also associated with worse neurocognitive performance (phonemic fluency: β=1.40 p=0.026; letter-number: β=0.69, p=0.07). Higher depression BSI scores were correlated with lower phonemic fluency (r=-0.13, p=0.007).

Discussion: Our results suggest that *NRN1* gene variability has a role in the development of depressive symptoms. Moreover, the association found between *NRN1* and cognitive performance suggests also a role of this gene modulating the cognition in a general population sample. This adds evidence about the pleiotropic effect of *NRN1*, a gene with multiple roles in neurodevelopment and synaptic plasticity.

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5

MIR137, ZNF804A AND *CACNA1C* GENES AND THE RISK FOR PSYCHOTIC DISORDERS: A FAMILY-BASED ASSOCIATION STUDY

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Background:

MicroRNAs (miRNAs) are non-coding endogenous single-stranded RNAs that regulate gene expression. Recent GWAS studies have identified *MIR137* as a candidate gene for schizophrenia (SZ), via association with an intronic single nucleotide polymorphism (SNP), rs1625579 (Ripke et al. 2011, 2014). Moreover, MIR137 has been validated as a regulatory factor of *ZNF804A* and *CACNA1C*, which are genes also associated to SZ (Wright, et al., 2013). We aimed to analyze the association of these genes and the risk for schizophrenia and psychotic disorders by means of a family-based study.

Methods: The sample comprised 616 individuals, including 172 patients with psychotic disorders, 311 parents and 173 siblings. DNA was extracted from saliva samples and three SNPs were genotyped: *MIR137* (rs1625579), *ZNF804A* (rs1344706) and *CACNA1C* (rs1024582), using Taqman 5' exonuclease assays. Transmission Disequilibrium Test (TDT) was conducted with PLINK.

Results: The genotype frequencies of the three SNPs were in Hardy-Weinberg equilibrium. TDT analyses showed a trend for transmission distortion of SNP rs1625579 at *MIR137* (χ^2=3.556 p=0.059), with the allele G being under-transmitted from parents to the affected offspring (i.e. protective factor). No association was detected for *ZNF804A* or *CACNA1C*.

Discussion: Our results point towards the implication of *MIR137* in psychotic disorders, suggesting that transcriptional or posttranscriptional regulatory mechanisms in which miR-137 is involved could underlie these disorders. This supports previous GWAS findings. Interestingly, it has been described that the T allele of rs1625579 is associated with hyperactivation of the dorsolateral prefrontal cortex, which is considered a risk phenotype for SZ (van Erp et al., 2014). Further studies are required to confirm and extend these findings.

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6
NOVEL MODE OF DNA RECOGNITION BY HMG PROTEINS

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HMGB proteins are members of the High Mobility Group (HMG) proteins. They are architectural proteins that affect cellular functions by modulating chromatin structure and thereby gene expression in eukaryotic cells. HMGB1 is considered the archetypal member of the HMGB family. It participates in the regulation of transcription, chromatin remodeling, recombination and DNA repair. In an extracellular role, HMGB1 is a danger signal in inflammatory conditions, including autoimmunity and cancer. HMGB1 contains two DNA binding domains, box A and box B, which have little sequence specificity but remarkable abilities to underwind and bend DNA through interacting with the minor groove of DNA.

Here we will present the structure of two HMGB1 box A domains bound to an AT-rich DNA fragment determined by X-ray crystallography at 2 Å resolution. Both of them collaborate in an unusual configuration where Phe37 of both box A domains stack together and intercalate the same CG base pair generating highly kinked DNA. This is a novel mode of DNA recognition for HMGB proteins and reveals a mechanism by which structure-specific HMG boxes kink and underwind linear DNA.

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7

STRUCTURAL ANALYSIS OF CONDENSED METAPHASE CHROMOSOMES BY SYNCHROTRON SMALL-ANGLE X-RAY SCATTERING

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TEM images of partially denatured chromosomes obtained using different procedures showed that bulk chromatin in chromosomes is organized forming multilayered plate-like structures. This planar structure was studied using cryo-EM, electron tomography, and AFM imaging and force spectroscopy in aqueous media (1). The results obtained suggested that nucleosomes in the plates are irregularly oriented, and that the successive layers are interdigitated (layer thickness 5-6 nm), presumably allowing face-to-face interactions between nucleosomes of adjacent layers. Multilayer plates can be self-assembled from chromatin fragments obtained by micrococcal nuclease digestion of metaphase chromosomes (2), and it has been suggested (3-5) that metaphase chromosomes could be self-organizing liquid crystal structures formed by many stacked layers of chromatin oriented perpendicular to the chromosome axis. We have used the NCD beamline of ALBA Synchrotron to study the internal structure of native chromosomes. Sediments containing chromosomes from human (HeLa) cells under different conditions were placed in plastic capillaries and were exposed to X-rays for 20-80 s. The typical peaks at ~2.8 and ~3.7 nm corresponding to the internal nucleosome structure were observed in all samples. The peaks at ~11 and ~30 nm corresponding, respectively, to the distances between parallel nucleosome columns and laterally packed 30-nm fibers were absent or showed very low intensities. Under all conditions containing structuring cations, and in particular under metaphase ionic conditions (17 mM Mg^{2+}, 120 mM K^{+}, 20 mM Na^{+}), a peak centered at 6 nm is prominent. This broad peak can be correlated with the short-range repetition of the ~6 nm distance between nucleosomes (face-to-face interactions) and between stacked layers.

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8
OPTIMIZATION OF PROTEIN COMPLEX ASSEMBLY IN VITRO

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Hetero-oligomeric complexes of unknown stoichiometry can be a challenge in understanding and reconstituting the natural complex assembly.

Once stable conditions for expressing and purifying an enzymatic active complex and individual complex-forming proteins are identified, stochiometry can be identified using Size-Exclusion-Chromatography (SEC) combined with Multi-Angle-Light-Scattering (MALS).

Furthermore, cofactors can be screened using Differential-Scanning-Fluorimetry and be used to stepwise reconstitute the hetero-oligomeric complex in vitro, verified by SEC and enzymatic assays.

We will present a case where all this techniques have been applied.

9

SEARCHING SURFACE MUTATIONS WITH HIGHER THERMOSTABILITY IN THE PROKARYOTIC LAT TRANSPORTER ASC-LIKE. TOWARDS THE STRUCTURE OF A LAT TRANSPORTER

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One of the definitive challenges in membrane proteins crystallization is the improvement of the crystals. These proteins have to be crystallized with detergents and, sometimes, with lipids. These special conditions often allow poor diffracting crystals. Mutagenesis is widely used in many kinds of proteins to improve packing properties. For several membrane proteins mutations of the surface residues are reported to increase the thermostability and to allow high resolution diffracting crystals. In this sense, we are searching for more thermostable mutants of a prokaryote homolog of the LAT transporters, Asc-like. Using an homology based model, we selected all residues predicted to be in contact with the phospholipids in the plasma membrane. Then we mutated each of these 176 residues to alanine and we set up a small scale protocol to test the thermostability with respect of the wild-type protein. We have identified three thermostable mutants. Combinations of these mutations have been tested and the most thermostable mutant is being used to improve diffracting crystals.

In parallel, we also searched for Asc-like inhibitors. We have shown that both prokaryotic and human Asc-1 transporters have identical selectivity for substrates and particular inhibitors. Using non-translocable inhibitors would aid in the generation of high quality crystals.

10

HIV-1 PROMONOCYTIC AND LYMPHOID CELL LINES AS PROPER IN VITRO MODELS OF IN VIVO MITOCHONDRIAL LESION

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Background: An altered mitochondrial function and apoptosis may affect the defense capacity of the organisms to face HIV-infection, contributing to establish chronically-infected patients. The validation of an *in vitro* model reproducing characteristic mitochondrial and apoptotic *in vivo* lesion would lead to the establishment of a platform for potential strategies to deal with the HIV-infection process.

Objective: To develop an *in vitro* model using chronically HIV-infected promonocytic and lymphoid cells: U1 and the same uninfected line U937; ACH2 and the same uninfected cell line CEM.

Methods: Mitochondrial DNA (mtDNA) content was analyzed by rt-PCR, mitochondrial complex-IV (CIV) enzymatic activity was spectrophotometrically measured. Mitochondrial and nuclear encoded subunits II/IV of cytochrome-c-oxidase (COXII/COXIV), and mitochondrial apoptotic events (VDAC-1/ß-actin content) were quantified by western blot, mitochondrial mass was assessed spectrophotometrically (citrate-synthase). Mitochondrial membrane potential and advanced apoptotic and necrotic events were measured through flow cytometry.

Results: mtDNA 57.67 % depletion ($p<0.01$) was found in U1 promonocytic cell line, compared with U937 controls. This was reflected by a significant 77.43 % decrease of mitochondrial CIV ($p<0.01$). COXII/COXIV subunits and VDAC-1/ß-actin contents were decreased in both chronically HIV-infected promonocytic and lymphoid cells ($p<0.005$). Additionally, U1 cells and ACH2 cells tended to lower levels of depolarized membranes than controls.

Conclusions: Both *in vivo* and in the present *in vitro* modeling, mitochondrial and apoptotic pathways are altered due to HIV-infection. This may contribute to perpetuate the chronicity of the infection. We provide a platform to test novel apoptotic and specially mitochondrial therapeutic options.

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MITOCHONDRIAL AND APOPTOTIC IN VITRO MODELLING OF DIFFERENTIAL HIV-1 PROGRESSION AND ANTIRETROVIRAL TOXICITY

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Background: *Ex vivo* analysis of mitochondrial function may reveal HIV progression and the impact of antiretrovirals. We propose a mitochondrial and apoptotic *in vitro* model using Jurkat T cells incubated with plasma.

Objectives: The aims of this study were to evaluate mitochondrial and apoptotic lesions in this model in relation to HIV progression, and to assess the effect of one year of standard non-thymidine-containing antiretroviral therapy.

Methods: This was a cross-sectional comparison among three age- and gender-matched groups (n=19×3): healthy non-HIV-infected participants, HIV-infected long-term non-progressors (LTNPs) and standard antiretroviral-naive chronically infected patients [standard progressors (Sps)], longitudinally evaluated before (Sp1) and after (Sp2) one year of efavirenz+tenofovir+emtricitabine therapy. We analysed mitochondrial DNA content by RT-PCR, mitochondrial function by spectrophotometry, mitochondrial protein synthesis by western blot analysis, mitochondrial dynamics by western blot analysis (MFN2), apoptotic transition pore formation by western blot analysis (VDAC1) and mitochondrial membrane potential and annexin V/propidium iodide fluorescence by flow cytometry.

Results: There was a decreasing non-significant trend towards lower mitochondrial parameters for HIV-infected values with respect to uninfected control reference values. HIV progression (LTNP versus Sp1) was associated with decreased mitochondrial genetic, functional and translational parameters, which partially recovered after treatment intervention (Sp2). Mitochondrial fusion showed a trend to decrease non-significantly in Sp patients compared with LTNP patients, especially after therapy. All apoptotic parameters showed a trend to increase in Sp1 with respect to LTNP, followed by recovery in Sp2.

Conclusions: We proposed an in vitro model for mitochondrial and apoptotic assessment to test the effects of HIV infection and its therapy, resembling *in vivo* conditions. This model could be useful for clinical research purposes.

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