phdRetreat

IRB Barcelona
14th 15th of November

35 Br eaking science together

IRB BARCELONA
INSTITUTE FOR RESEARCH IN BIOMEDICINE

1st IRB Barcelona PhD Retreat
Hotel Campus UAB, Cerdanyola del Vallès
14-15th November 2014

Contents

Welcome by the Organizers

It is our great pleasure to welcome you to the 1st IRB Barcelona PhD Retreat, which will be held from November 14th to 15th of November in Barcelona at "Hotel Campus UAB".

The main motivation of this Retreat is to promote exchange of knowledge between young researchers within the IRB PhD community. There will be not only talks and poster sessions done by and for students but also we will have time for talks from former IRB Barcelona PhD Students and leisure time to strengthen links between the community.

We would like to welcome and thank the 11 invited students coming from three excellent research institutes (Nencki Institute, RIMLS Institute and Università di Trento) for participating in the retreat. We hope we will establish solid collaborations between our research institutes.

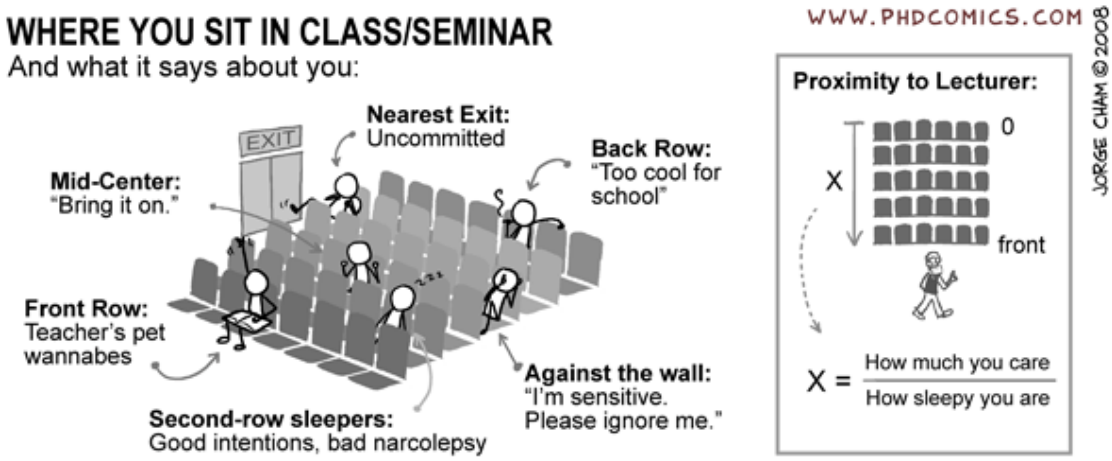
To encourage good scientific work done by PhD-students, prizes will be awarded to the best poster (€100) and best talk (€200).

We consider that this is a good opportunity for IRB Barcelona to improve the PhD programme in order to train even better and more prepared young scientists.

All we want is a great scientific institute and we do not doubt that this initiative will push IRB Barcelona in that direction.

We hope you enjoy the Retreat!

Retreat Organizing Team
Institute for Research in Biomedicine (IRB Barcelona)



Useful information

Dear Guests,

Welcome to the PhD Retreat and UAB Campus.

Symposium Badge

Please wear your name at all times, this is to promote networking and to assist staff in identifying you.

Wireless Connection

Wireless Internet access is available throughout the building.

Presentations

If you are an invited speaker or short talk, please bring a copy of your presentation in pdf and check before your session that the organizers have received a copy.

Poster Walks instructions

Each Poster Walk will be integrated by a moderator (who will guide the poster talk), the presenters (people with poster to present) and listeners (people without poster).

Presenters (3 people in each group) are requested to do a 10 minutes presentation of the poster followed by 5 minutes questions by the people integrating the Group. Once the fist presenter had presented the poster, the Group has to move to the next poster.

The moderator (1 or 2 in each group) has to control the timing of presentation and moderate the questions. In each group, the moderator (or moderators) is one student that presents a talk in the Retreat.

The function of the listeners (3 people in each group) is to pay attention of each poster presentation and make at least one question for each poster.

Meals

Coffee breaks will be served in front of the poster room just in front of the Auditorium. Lunch and dinner consist in a buffet in the same hotel.

Social Networks

#IRBRetreat

If you have any queries or comments, please do not hesitate to contact a member staff, which will be pleased to help you.

Kind regards,

Retreat Organizing Team
Institute for Research in Biomedicine (IRB Barcelona)

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Schedule Summary

	November	
	Friday 14th	Saturday 15th
08:30-09:00	Registration	Breakfast
09:00	Welcome and Session1 (Talks and Carlos Garcia)	Session 4 (Talks)
10:00		Poster Session
11:00	Coffee Break	Coffee Break
11:30	Session 2 (Talks and Georgina Sorrosal de Luna)	Session 5 (Talks and David Vilchez)
12:45		Closure
13:00	Lunch	Lunch
14:30	Check-in / Hang posters!	
15:00	Workshop (Helena González)	Fun Sport
16:30	Coffee Break	
17:00	Session 3 (Talks)	
18:00	Poster Walk	
19:00	Free Time	
20:00	Dinner	
21:00	Bar time!	

Retreat Programme

Friday 14th November

8:30 – 9:00 Registration
9:00 – 9:15 Welcome by Raúl Mendez, IRB Group Leader and head of the PhD Committee

Session 1

9:15 – 10:00 Invited speaker, Dr. Carlos Garcia-Echeverria, Global Head of Small Molecule Drug Discovery at Sanofi-Aventis.

"Unleashing pharma drug discovery"

Chair: Julia Garcia, PhD Student.

10:00 – 10:15 1st Talk. **Josep Garcia Garcia**, Chemistry and Molecular Pharmacology Programme, IRB Barcelona. *"New strategies based on nanosystems to facilitate the crossing through biological barriers".*

10:15 – 10:30 2nd Talk. **Giorgia Testoni**, Molecular Medicine Programme, IRB Barcelona. *"The many facets of glycogenin".*

10:30 – 10:45 3rd Talk. **Pedro Sfriso**, Structural and Computational Biology Programme, IRB Barcelona. *"Exploring protein conformational space hand in hand with evolution".*

10:45 – 11:00 4th Talk. **Aleix Bayona**, Cell and Developmental Biology, IRB Barcelona. *"Drosophila Linker histone H1 prevents DNA replication stress".*

11:00 – 11:30 Coffee Break

Session 2

11:30 – 11:45 5th Talk. **Pau Rocas-Alonso,** Chemistry and Molecular Pharmacology Programme, IRB Barcelona. *"Installing multifunctionality on titanium with RGD-decorated polymeric nanocapsules: Towards new osteointegrative therapies".*

11:45 – 12:00 6th Talk. **Jordina Guillén,** Molecular Medicine Programme, IRB Barcelona. *"CPEB4 hyperphosphorylation is required for its function in the meiotic cell cycle".*

12:00 – 12:15 7th Talk. **Anabel-Lise le Roux,** Chemistry and Molecular Pharmacology Programme, IRB Barcelona. *"Biophysical Studies of Myristoylated Unique and SH3 Domains of Src Kinase and their Interactionwith Lipid Membranes".*

12:15 – 13:00 Invited speaker, Dr. Georgina Sorrosal de Luna, Innovation Manager at VHIR.

"Bio- options" beyond the bench

Chair: Marco Milán, IRB Group Leader.

13:00 – 14:30h Lunch

14:30 – 15:00 Time for: Check in - Hang Posters

15:00 – 16:30 Workshop, Dr. Helena Gonzalez, Public Engagement and Science Education Office at IRB Barcelona.

"Science Communication Workshop"

Chair: Rosa María Ramírez, PhD Student

16:30 – 17:00 Coffee Break

Session 3

17:00– 17:15 8th Talk. **Francisco Freixo**, Cell and Developmental Biology, IRB Barcelona. *"Novel roles for the mitotic kinase Nek7 in post-mitotic neurons".*

17:15– 17:30 9th Talk. **Giulio Chiesa,** Chemistry and Molecular Pharmacology Programme, IRB Barcelona. *"Androgen receptor oligomerization in spinal bulbar muscular atrophy: a study at the residue level".*

17:30– 17:45 10th Talk. **Jarosław Walczak,** Nencki Institute of Experimental Biology, Warsaw, Poland. *"Mitochondrial stress in sporadic and familial form of ALS"*.

17:45– 18:00 11th Talk. **Simone Pieretti,** Structural and Computational Biology Programme, IRB Barcelona. *"Structural characterization of the DNA uptake machinery in gram-positive bacteria"*.

18:00– 19:00 Poster Walk

19:00– 20:00 Free time

20:00– 21:00 Dinner

21:00– 24:00 Bar time!

Saturday 15th November

Session 4

09:00 – 09:15 12th Talk. **Salvador Pérez-Montero,** Cell and Developmental Biology, IRB Barcelona. *"The Drosophila histone H1 variant, dBigH1, regulates zygotic genome activation and plays an essential role in germline development".*

09:15 – 09:30 13th Talk. **Sílvia Vilaprinyó-Pascual**, Chemistry and Molecular Pharmacology Programme, IRB Barcelona. *"Low-n Aβ oligomers: globular structure and well-defined cross-linked mimics".*

09:30 – 09:45 14th Talk. **Malgorzata Hanna Hall,** Nencki Institute of Experimental Biology, Warsaw, Poland. *"One of the components of neuronal nuclear architecture: PML nuclear bodies"*

09:45 – 10:00 15th Talk. **Elena Álvarez-Marimon**, Molecular Medicine Programme, IRB Barcelona. *"Structural bases for the interaction and stabilization of the human amino acid transporter LAT2 with its ancillary protein 4F2hc".*

10:00 – 11:00 Poster Session

11:00 – 11:30 Coffee Break

Session 5

11:30 – 11:45 16th Talk. **Enrique J Arenas,** Oncology Programme, IRB Barcelona. *"RARRES3 suppresses breast cancer lung metastasis by regulating adhesion and differentiation"*

11:45 – 12:00 17th Talk. **Benjamí Oller-Salvia**, Chemistry and Molecular Pharmacology Programme, IRB Barcelona. *"FROM BEE VENOM TO BLOOD-BRAIN BARRIER SHUTTLES: apamin derivatives are capable of transporting antibodies across the barrier"*

12:00 – 12:45 Invited speaker, **Dr. David Vilchez**, Principal Investigator at CECAD.

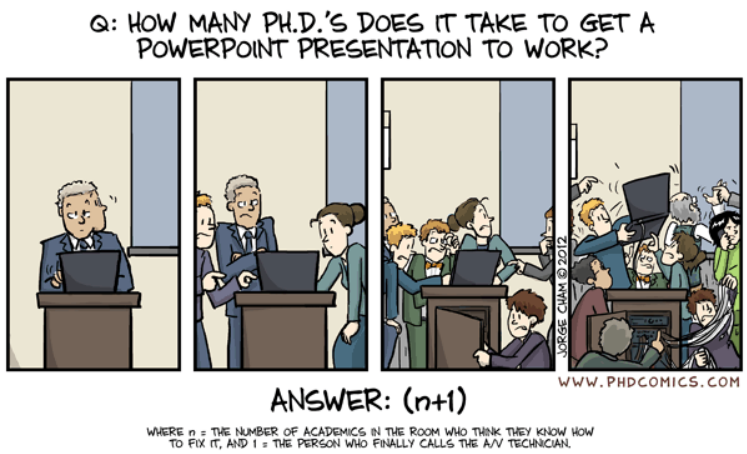
"Proteostasis of aging and stem cells"

Chair: Marion Salzer, PhD Student.

12:45 – 13:00 Closure by Raúl Mendez, IRB Group Leader and head of the PhD Committee

13:00 – 14:30 Lunch

14:30 – 16:30 Fun Sport!



Invited Speakers

Dr. Carlos Garcia-Echeverria

Global Head of Small Molecule Drug Discovery at Sanofi-Aventis.

Carlos Garcia-Echeverria, Ph.D., is the current Global Head of Small Molecule Drug Discovery at Sanofi-Aventis. Before, he has served as Global Head of Oncology Medicinal Chemistry & Pharmacology and Deputy Global Head of Oncology Drug Discovery and Preclinical Development at Sanofi-Aventis since April 2010.

From 1993 to 2010, Dr. Garcia-Echeverria held several positions of increasing responsibility at the Novartis Institutes for BioMedical Research. In his last position as Executive Director of Oncology Drug Discovery, he was responsible for managing the late drug discovery programs –lead to first-patient-first-visit- at the Novartis Oncology Research site in Basel, Switzerland. His research activities have been mainly focused on the identification and development of inhibitors of protein and lipid kinases, proteolytic enzymes and antagonists of intracellular protein-protein interactions. Dr Garcia-Echeverria has made significant contributions to the identification and development of targeted cancer therapies and has advanced seven drug candidates to Phase I/II clinical trials.
He is an inventor on 30 patents, and has published more than 150 peer-reviewed articles, book chapters and review papers. Dr Garcia-Echeverria received his Ph.D. in organic chemistry from the University of Barcelona (Spain) in 1993, doing his thesis work in the group of Prof. Ernest Giralt.

He is Senior Editor of “Chemical Biology & Drug Design” and board member of “Drug Design Reviews-Online”, “Expert Opinion on Therapeutic Targets”, “Journal of Peptide Research and Therapeutics”, “Current BioData”, “Recent Patent Reviews on Anti-cancer Drug Discovery”, and “The Open Cancer Journal”. Dr Garcia-Echeverria received the Leonidas Zervas Award from the European Peptide Society in 2006.

Selected Publications:

- Carry JC, García-Echeverría C. (2013) Inhibitors of the p53/hdm2 protein-protein interaction-path to the clinic. Bioorg Med Chem Lett. 23(9):2480-5.
- García-Echeverría C. (2010) Allosteric and ATP-competitive kinase inhibitors of mTOR for cancer treatment. Bioorg Med Chem Lett. 20(15):4308-12.
- Gao Z, García-Echeverría C, Jensen MR. (2010) Hsp90 inhibitors: clinical development and future opportunities in oncology therapy. Curr Opin Drug Discov Devel. 13(2):193-202.

At 09:15 on Friday 14th,

Dr. Carlos Garcia-Echeverria

"Unleashing pharma drug discovery"

Abstract

Faulty identification and validation of therapeutic targets, inadequate preclinical lead characterization in models that poorly mimic human diseases, and lack of suitable and robust translational medicine biomarkers are key contributors to the unprecedentedly high clinical attrition rate of investigational drugs despite the increasing numbers of candidates entering clinical trials and burgeoning drug discovery expenditures in the public and private biomedical sectors. The discovery and development of clinically effective and safe drug depends critically upon experimental and *in silico* approaches capable of providing high-impact data on the efficacy and safety profile of hits, leads and pre-candidates. The in-depth evaluation of candidate compounds in biologically and pharmacologically relevant models should enable reliable lead identification, compound differentiation and successful translation of research output into clinically useful new therapeutics. This educational presentation will cover different parts of the drug discovery value chain and technologies that are used in contemporary drug discovery. Representative examples will be selected to illustrate how these techniques, when applied in an informed manner congruent with the targeted disease and intended mechanism of action of the drug, can serve as valuable enablers of drug discovery and potentially contribute to reducing the current, unacceptably high rates of compound clinical failure.

Dr. Georgina Sorrosal de Luna

Innovation Manager at VHIR

Georgina Sorrosal de Luna obtained her PhD from IRB in 2010, where she studied the secreted serine protease-like protein Scarface on Drosophila embryo under the direction of Marco Milán.

She then decided to focus her career into Science management. From 2010-2012 she worked at Associació de Biotecnòlegs de Catalunya (ABSTEC), a non-profit association, organizing different congresses and activities to link research & business in the biotech and biomedical field. In 2011, she joined Advancell biotech company, focused in drug development, where she worked as a Project Manager leading a clinical trial in multiple sclerosis for 3 years. Currently Georgina has a position as Innovation Developer at Vall d'Hebron Institute of Research (VHIR).

Selected Publications:

- Sorrosal G, Pérez L, Herranz H, Milán M. (2010) Scarface, a secreted serine protease-like protein, regulates polarized localization of laminin A at the basement membrane of the Drosophila embryo. EMBO Reports 11(5):373-9.

- Bejarano F, Luque C M, Herranz H, Sorrosal G, Rafel N, Pham T T, Milán M. (2008) A gain-of-function suppressor screen for genes involved in dorsal-ventral boundary formation in the Drosophila wing. Genetics. 178(1):307-23.

At 12:15 on Friday 14th,

Dr. Georgina Sorrosal de Luna

"Bio- options" beyond the bench

Abstract

Finishing a PhD is always challenging and a bit scaring. What to do next? Going for a post-doc is not the only option, as there are plenty of exciting jobs still related to Science. Georgina will tell us her experience in different jobs related to the Science management both in the private and public sector.

Dr. David Vilchez

Principal Investigator at CECAD

David Vilchez obtained his PhD from IRB in 2008. Working at Joan Guinovart´s lab he studied Lafora Disease and brain glycogen. After he worked at the Salk Institute in California and at the University of California Berkeley, where his studies focused on aging in C. elegans. From his work at Dillin´s lab he successfully publishes two Nature papers.

Actually David talented career continues as a Principal Investigator at the Cologne Excellence Cluster on Cellular Stress Responses in Aging-Associated Diseases (CECAD). Where he is interested in studying how human embryonic stem cells maintain the integrity of their proteome.

Selected Publications:

- Vilchez D, Boyer L, Lutz M, Merkwirth C, Morantte I, Tse C, Spencer B, Page L, Masliah E, Berggren WT, Gage FH and Dillin A (2013) FOXO4 is necessary for neural differentiation of human embryonic stem cell. Aging Cell 12 (3): 518-522.

- Vilchez D, Simic M, Dillin A (2013) Proteostasis and aging of stem cells. Trends in Cell Biology

- Vilchez D, Boyer L, Morantte I, Lutz M, Merkwirth C, Joyce D, Spencer B, Page L, Maslias E, Berggren WT, Gage FH and Dillin A (2012). Increased proteasome activity in human embryonic stem cells is regulated by PSMD11. Nature 489 (7415): 304-308.

- Vilchez D, Morantte I, Liu Z, Douglas PM, Merkwirth C, Rodrigues AP, Manning G and Dillin A (2012). RPN-6 determines C. elegans longevity under proteotoxic stress conditions. Nature 489 (7415): 263-268.

At 12:00 on Saturday 15th,

Dr. David Vilchez

"Proteostasis of aging and stem cells"

Abstract

While it is has long been noted that genome stability is a central function required for survival of stem cells, the role of proteostasis has not been explored. With the asymmetric divisions invoked by stem cells, the passage of damaged proteins to daughter cells can destroy the resulting lineage of cells and possibly accelerate the aging process. Furthermore, the possible retention of damaged proteins by the stem cell can result in diminished stem cell function and possibly premature aging. Therefore, a firm understanding of how stem cells maintain their proteostasis is of central importance. Because experiments with mammalian embryonic stem cells have clearly demonstrated their capacity to replicate continuously in the absence of senescence, we hypothesize that these cells could provide a novel paradigm to study the regulation of proteostasis and its demise in aging. We have recently described that human embryonic stem cells (hESCs) exhibit high proteasome activity compared to their differentiated counterparts. This enhanced proteasome activity is necessary for hESC function. Furthermore, we have uncovered that PSMD11/*rpn-6*, a key proteasomal subunit, is required for this activity and its mode of regulation and conservation in the aging process of the invertebrate *C. elegans*. Our findings established RPN-6 as a potent regulator of proteasome activity that alleviates the deleterious effects associated with aberrant protein aggregation, providing a powerful candidate to correct proteostatic deficiencies in disorders such as HD. Furthermore, our results in *C. elegans* led us to find that FOXO4, a transcription factor associated with longevity, regulates proteasome activity in hESCs and is necessary for hESCs differentiation into neural cells. Therefore, our results established a novel regulation of proteostasis in hESCs that links longevity and stress resistance in invertebrates with hESC function and identity.

Workshop

Dr. Helena Gonzalez

Public Engagement and Science Education Office at IRB Barcelona

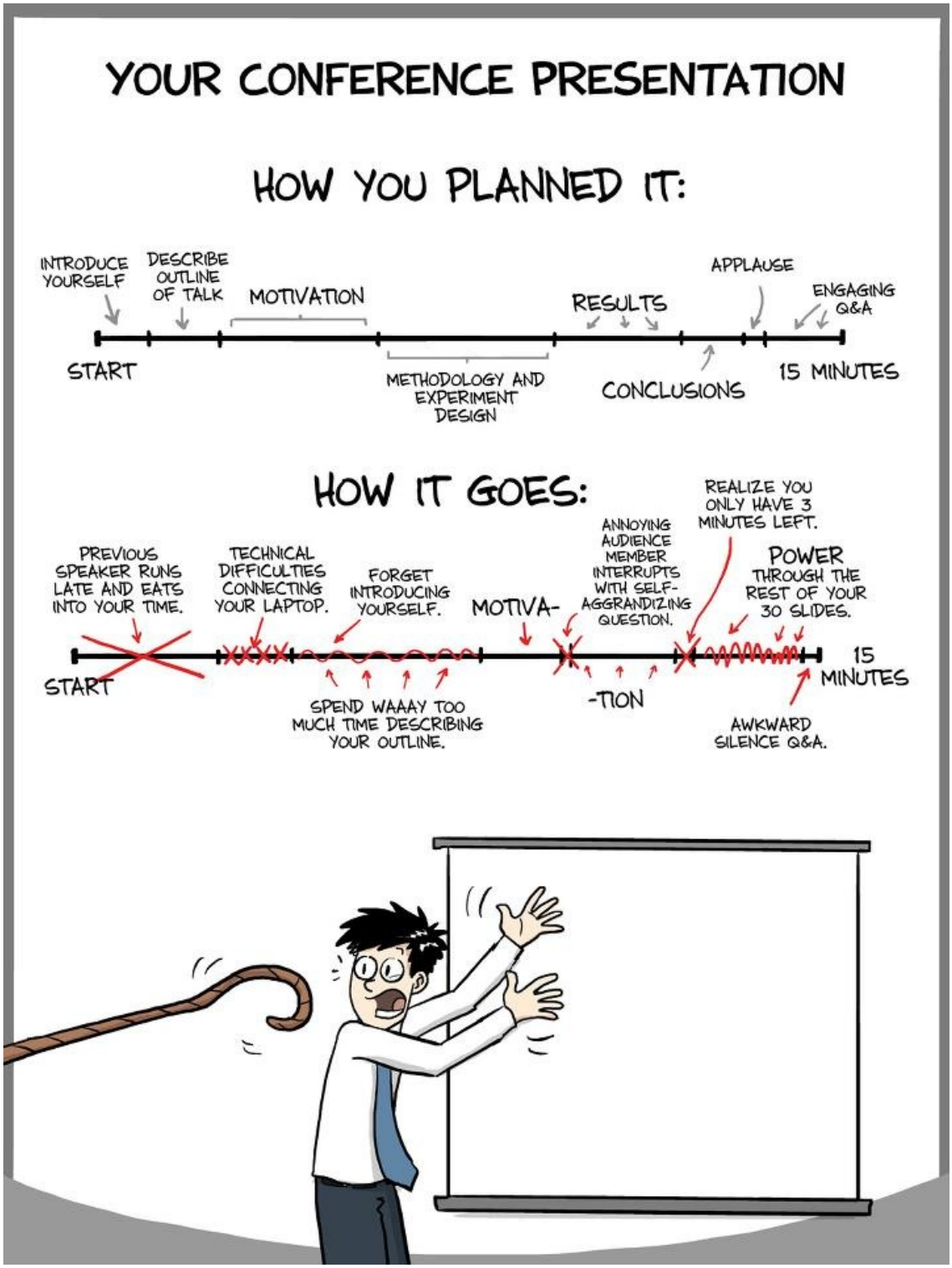
Helena González defended her PhD in march 2014, with the project: “The role of TLKs in Genome Stability and Cancer development” developed in the lab of Travis Stracker, IRB. During this period Helena participated in most of the communication activities in the IRB. She’s also cofounder of Clowntífics (2011) and The Big Van Theory (2013), social enterprises that aim to communicate science in the most unexpected places: theaters, pubs, discos, social centers... always using humor as a powerful tool to connect with the audience.

Nowadays she is Public Engagement and Science Education Officer at IRB.

At 15:00 on Friday 14th,

Science Communication Workshop

The Public Engagement and Science Education programme of IRB brings together various science outreach and communication activities with the following common objectives: to communicate the cutting-edge research done at the institute, to promote scientific vocation in young people and to promote scientific literacy among the general public.
As a PhD student you have a lot to offer to the society: IRB needs motivated researchers to develop engaging hands-on activities, run workshops for kids and teenagers, to provide teachers with the tools they need to instill a passion for science in their students, to encourage critical thinking in the general public... we need new hands and new ideas to find creative ways to explore scientific ideas with public audiences. And we are starting now!


YOUR CONFERENCE PRESENTATION
HOW YOU PLANNED IT:
INTRODUCE YOURSELF
DESCRIBE OUTLINE OF TALK
MOTIVATION
RESULTS
APPLAUSE
ENGAGING Q&A
START
METHODOLOGY AND EXPERIMENT DESIGN
CONCLUSIONS
15 MINUTES
HOW IT GOES:
PREVIOUS SPEAKER RUNS LATE AND EATS INTO YOUR TIME.
TECHNICAL DIFFICULTIES CONNECTING YOUR LAPTOP.
FORGET INTRODUCING YOURSELF.
MOTIVA-
ANNOYING AUDIENCE MEMBER INTERRUPTS WITH SELF-AGGRANDIZING QUESTION.
REALIZE YOU ONLY HAVE 3 MINUTES LEFT.
POWER THROUGH THE REST OF YOUR 30 SLIDES.
START
SPEND WAAAY TOO MUCH TIME DESCRIBING YOUR OUTLINE.
-TION
15 MINUTES
AWKWARD SILENCE Q&A.

Talk 1

"New strategies based on nanosystems to facilitate the crossing through biological barriers"

Josep Garcia Garcia

Abstract

The last years, a lot of effort was done in order to optimize the synthesis of gold nanorods. Finally, the use of hydroquinone was the key for obtaining an acceptable yield of rods (more than 90% of rods obtained from the synthesis). Once the synthesis was highly optimized and reproducible, surface modification was the next step. Here, in this report I'll explain how the surface of the rods was functionalized and some preliminary results from cell internalization. Apart from the rods, two other nanosystems were studied during the last year: insulin complexation with polyarginine peptide analogs in order to cross intestinal membrane and poly(lactic-co-glycolic acid) (PLGA) nanoparticles for crossing the blood brain barrier (BBB).

As previously mentioned, the rod surface has to be functionalized in order to internalize them into the cells. In our case, 4 different strategies were used:

1- The use of mercaptoundecanoic acid (MUA). The thiol group will form a covalent bond with the gold surface and in the other end a carboxylic group will serve us to couple a cell penetrating peptide (CPP).
2- The use of MUA and poly ethylene glycol (PEG). PEG will be used in order to prevent nanoparticles from aggregation and make them more water soluble.
3- The use of HS-PEG-COOH. Again, thiol groups will attach to the rod surface and CPP will be coupled to the carboxylic end. In this case, the reaction will take place in a solution containing K_2CO_3.
4- The use of HS-PEG-COOH. In this case, no salts were added to the solution.

After surface different rods functionalization, the peptide was added to the solution to finally obtain NRs-linker-peptide particles.
Preliminary results using octaarginine (r_8) as a CPP showed us which of the previously formulations were internalized into HeLa cells. In the picture we can observe fluorescence into cells obtained with a confocal microscopy. In this case, the strategy 3 was the best and with the other strategies no fluorescence could be observed into the cells. These results have to be repeated and the functionalization step has to be optimized in order to obtain better and conclusive results.

On the other hand, I was in Dublin during 10 weeks and other biological barrier was used in this case. The main objective was make some formulations mixing r_8 (the same CPP used to internalize the rods into the cell) and analogs with insulin. It is reported that these physical mixtures between insulin and some CPPs can improve the transport of insulin through the intestinal barrier. One model that could help us to observe this effect is the called Ussing chambers (picture on the right).

In these equipment, a piece of tissue is placed in the middle of two chambers (in our case, rat colon) and the compounds are added to the left chamber (donor chamber). After a period of time, an aliquot from the right chamber is collected (acceptor chamber) and the amount of compoud is quantified. In this maner, we can compare different peptides and see which one is a better transporter.

Finally, the last project I'm involved on is related with Friedreich's Ataxia. The aim of this project is the transport of DNA throgh the BBB. For this purpose, PLGA nanoparticles containig DNA will be synthesized. At this moment, the polymer with different blocks is optimized. Apart from PLGA, PEG and peptides will be attached to form a block co-polymer. This polymer will have the ability of encapsulate the DNA and the peptide will serve us to cross the BBB in this case.

Talk 2

"The many facets of glycogenin"

Giorgia Testoni, Maria del Mar García Rocha & Joan J. Guinovart

Institute for Research in Biomedicine (IRB Barcelona)

Abstract

Glycogen is synthetized by the cooperation of 3 enzymes: glycogenin (GYG), glycogen synthase (GS), and glycogen branching enzyme (GBE). The first step of this process is mediated by the action of GYG able to synthesize a short chain of glucose (up to 11 residues). GS uses this primer to elongate the polymer in a linear chain by α, 1-4, glycosidic linkage, and GBE contributes to generate α, 1-6, glycosidic branches. Glycogenin has a 35 amino acid-motif responsible for binding to GS, and on the other hand GS is able to bind to Gyg. This interaction seems to be indispensable for GS activity.

Our initial hypothesis was that in case of GYG depletion, glycogen synthesis was totally prevented. To elucidate the role of GYG interaction on the regulation of glycogen synthesis we created a transgenic mouse model lacking Gyg. Although the lethality of the animal was high (90%), the animals were unexpectedly able to synthesize glycogen in several tissues. We observed abnormal morphology of the heart and high content of glycogen in heart and muscle that might affect their functionality during development. The study of the surviving Gyg KO mice show that every tissue responds differently to the depletion of glycogen: liver maintained the normal level, muscle and heart glycogen increased drastically and brain showed reduced polysaccharide accumulation.

We have challenged the utilization of glycogen in cases of energy need in Gyg KO mice and our results elucidates whether the accumulation observed in muscle and heart are due to higher synthesis or lower degradation. Moreover we used hepatocytes as an ex vivo model to study glycogen mobilization analyzing the time course of glycogen turnover.

Furthermore we performed proteomics analysis to determine whether the lack of glycogenin is compensated by a substitute protein. Purifying glycogen from WT and Gyg KO tissues and removing all the proteins that are non-covalently bound to the granule and we obtained two possible candidate proteins able to replace glycogenin and create a covalent bound with the polymer. Our aim is to demonstrate the mechanism by how the candidate substitute protein acts and also we will determine the role of glycogenin in terms of regulator of glycogen synthesis (acting on GS activity or others proteins) and its participation on cellular distribution of the polysaccharide (due to its actin binding site).

This discovery opens up a new line of investigation based on the role of glycogenin on the regulation of the synthesis of glycogen and the pursuit of possible other proteins involved in the glycogen initiation process.

Talk 3

"Exploring protein conformational space hand in hand with evolution"

Pedro Sfriso[1,2], Miquel Duran-Frigola[1,2], Patrick Aloy[1,2] & Modesto Orozco[1,2]

[1]Institute for Research in Biomedicine (IRB Barcelona)[2]Joint BSC-CRG-IRB Research Program in Computational Biology, Barcelona, Spain

Abstract

Many biological functions of proteins such as mechanic work, signal transduction or enzymatic activity are modulated by their flexibility. Structural databases show increasing number of proteins having alternative structures depending on external factors (such as crystallization conditions, chemical modifications, presence of ligands, etc). This probes the existence of conformational transitions in proteins, but giving no information on how such transitions happen. Despite recent advances, experimental techniques can rarely describe how the transitions occur with sufficient detail. This forces the use of computational techniques by themselves or in combination with experiments. Standard simulation packages suffer from the huge gap between the elementary motions in physical simulations and the biological time scale, making the direct study of conformational transitions unaffordable.

In this scenario, we present a new computational method that is able of obtaining protein dynamic information based on coarse-grained models and discrete molecular dynamics[1]. We developed a hybrid method that accelerates physical simulation based on sequence coevolution contacts[2,3]. The idea behind the method is that the sequence is optimized for maintaining not one but all protein alternative configurations. The method reveals protein conformational space given that there is at least one structure deposited in PDB and a minimum number of homologous sequences are available.

References

- (1) Sfriso, P.; Hospital, A.; Emperador, A.; Orozco, M. Exploration of Conformational Transition Pathways From Coarse-Grained Simulations. Bioinformatics 2013, 29, 1980– 1986.
- (2) de Juan, D.; Pazos, F.; Valencia, A. Emerging Methods in Protein Co-Evolution. Nat Rev Genet 2013, 14, 249–261.
- (3) Morcos, F.; Jana, B.; Hwa, T.; Onuchic, J. N. Coevolutionary Signals Across Protein Lineages Help Capture Multiple Protein Conformations. Proceedings of the National Academy of Sciences 2013, 110, 20533–20538.

Talk 4

“Drosophila Linker histone H1 prevents DNA replication stress”

Aleix Bayona

Abstract

Histone H1 is an intrinsic component of chromatin, whose important contribution to chromatin structure is well-established in vitro. Little is known, however, about its functional roles in vivo. Here, we have addressed this question in Drosophila, a model system offering many advantages since it contains a single dH1 variant. For this purpose, RNAi approaches were used to efficiently deplete dH1 in flies and SL2 cells.
In flies, expression-profiling shows that dH1 depletion affects expression of a relatively small number of genes in a regional manner. Furthermore, depletion up-regulates inactive genes, preferentially those located in heterochromatin, while active euchromatic genes are down- regulated, suggesting that the contribution of dH1 to transcription regulation is mainly structural, organizing chromatin for proper gene-expression regulation. Up-regulated genes are remarkably enriched in transposons. In particular, R1/R2 retrotransposons, which specifically integrate in the rDNA locus, are strongly up-regulated. Actually, depletion increases expression of transposon-inserted rDNA copies, resulting in synthesis of aberrant rRNAs and enlarged nucleolus. Concomitantly, dH1-depleted cells accumulate extra-chromosomal rDNA, show increased γH2Av content, stop proliferation and activate apoptosis, indicating that depletion causes genome instability and affects proliferation. H1 depletion in wings results in atrophic wings, DNA damage, and apoptosis that can be rescued by p35 overexpression. Wing growth could also be recovered mainly by knocking down ATM (Tefu) and also by knocking down Chk1 (grp) and Chk2 (loki) although to a lesser extent. Coexpression of a p53 dominant negative form did not rescue wing size suggesting that depletion of histone H1 causes p53- independent apoptosis.
Similarly, dH1 depletion also leads to an accumulation of γH2Av content in SL2 cells. Further analysis of this increase in γH2Av showed that it mainly happens during the S phase and is DNA replication-dependent.

Talk 5

"Installing multifunctionality on titanium with RGD-decorated polymeric nanocapsules: Towards new osteointegrative therapies"

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Introduction

Installing multifunctionality on biomaterials is a powerful approach to increase their bioactivity. In particular, this strategy could be applied to metallic substrates to improve and accelerate osteointegrative processes 1. The aim of this work was to introduce an innovative methodology to synthesize polyurethane- polyurea nanocapsules (PUUa NCs) functionalized with cyclic RGD peptides (Fig. 1), which show great cell- binding activity and allow the encapsulation of hydrophobic drugs. Different PUUa NCs RGD- decorated were coated on Ti surfaces, and their effect on the adhesion of osteoblast like cells investigated. Moreover, the encapsulation of roxithromycin and its antibacterial effects were studied.

Experimental Methods

The NCs were covalently immobilized on Ti samples via a two-step protocol. First, PUUa NCs were synthesized by successive polycondensations between diisocyanate and diamino/diol monomers, followed by 2an aqueous emulsification. Subsequently, PUUa free isocyanate groups were reacted with Ti amino groups. To this end, Ti samples were previously activated with oxygen plasma and aminosilanized. The presence and distribution of the capsules was analyzed by SEM and fluorescence microscopy. The influence on cell behaviour was investigated using SAOS-2 cells by cell adhesion assays. The encapsulation of roxithromycin was studied by HPLC, and evaluated in antimicrobial assays.

Results and Discussion

The functionalization of Ti with the NCs was optimized by using distinct crosslinking units until an homogenous particle distribution was observed by SEM and fluorescence microscopy. RGD-nanocapsules significantly improved the adhesion of Saos-2 cells compared to controls (Fig. 2). Moreover, the presence of the PUUa-RGD capsules supported very good levels of spreading, with clear actin filaments and cytoskeletal organization, and the formation of focal adhesions. Encapsulation of roxithromycin (antibacterial, anti- inflammatory) was also explored.

Conclusion

The immobilization of PUUa NCs functionalized with RGD peptides onto Ti is a feasible and very promising strategy to increase the bioactivity of Ti and accelerate osteointegrative processes. Encapsulation of distinct drugs using these systems opens new prospects for applications in biomaterials and regenerative therapies.

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Talk 6

"CPEB4 hyperphosphorylation is required for its function in the meiotic cell cycle"

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Abstract

The meiotic cell cycle is driven by translational activation of dormant maternal mRNAs stored in the oocyte. This translational activation occurs due to the cytoplasmic polyadenylation of mRNAs, which is driven by two members of the CPEB family of proteins, CPEB1 and CPEB4. While CPEB1 is responsible for the translational activation of mRNAs until the metaphase of the first meiotic division (MI), CPEB4 activates mRNAs from interkinesis to the metaphase of the second meiotic division (MII). The aim of this project is to understand how CPEB4 activity is regulated by phosphorylation during the meiotic cell cycle. In vitro kinase assays and two dimensional phospho- peptide maps show that there are specific phosphorylation events in every meiotic phase and that they are driven by at least two different kinases: p42MAPK and cdc2. Mass spectrometry analysis of in vitro phosphorylated xCPEB4 lead us to the identification of twelve phosphorylation sites in the N-terminal half of the protein. Interestingly, functional assays suggest that xCPEB4 is activated by the hyperphosphorylation of these twelve sites. Accordingly, a non-phosphorylable mutant of xCPEB4 is not able to rescue the phenotype that xCPEB4 antisense has on metaphase plate formation, while a phospho-mimetic mutant does. These results highlight the relevance that hyperphosphorylation has on xCPEB4 function.

Talk 7

"Biophysical Studies of Myristoylated Unique and SH3 Domains of Src Kinase and their Interactionwith Lipid Membranes"

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Abstract

Src is member of the non receptor tyrosine kinase Src family, consisting in nine members that show similar modes of regulation and act as important signaling intermediaries regulating a variety of outputs, such as cell proliferation, differentiation, apoptosis, migration, and metabolism[1]. NMR studies of the intrinsically disordered N-terminal domain of Src, called Unique Domain (USrc), points out important properties unravelling regulation capabilities of this domain on Src activity[2,3]. Src is anchored to the membrane at its N-terminal domain via a cooperative hydrophobic/electrostatic interaction with the membrane: the first amino acids of USrc are highly basic and interact with acidic phospholipids, and a myristoyl tail is added post translationally at its N-terminal by N-Myristoyl Transferase, (NMT), giving Src the capability of inserting in the lipid bilayer[4,5,6]. Another region of the Unique Domain is interacting with lipids, the Lipid Binding Region (ULBR), and the Unique Domain also interacts with its next folded domain, SH3[3]. This work aims at characterizing these interactions in the Unique or the Unique+SH3 domains of Src in presence of the myristoyl anchoring to models of lipid bilayers. The Myristoylated Unique+SH3 domains of Src (MyrUSH3) has been obtained by coexpression of NMT and USH3 in E.Coli[7]. Triton wash extraction enables the purification of MyrUSH3. Surface plasmon resonance studies of MyrUSH3 binding to acidic liposomes confirmed the two- step mechanism of MyrUSH3 anchoring: a fast approximation to the lipid bilayer, followed by a slow insertion, whose kinetics can be characterized by using a secondary antibody detection. Myristoylated Unique Domain of Src (MyrUSrc) has been obtained by in vitro myristoylation and subsequent purification by HPLC. NMR studies of Myr-USrc binding to liposomes confirmed the binding of the ULBR to acidic phospholipids, and showed how this binding was enhanced if the protein was already anchored to the bilayer, and how it restricted the protein conformation. A construct of MyrUSrc followed by the GFP protein (MyrUGFP) has been obtained by the coexpression method and is used in an ongoing fluorescence microscopy study in the single molecule regime, to verify the monomeric state in the lipid environment, and to study the interaction of the ULBR to lipid bilayers, possibly docking the protein towards it. Constructs for optical tweezers experiments are ready, aiming at further characterizing the force of the ULBR interaction to lipid vesicles.

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Talk 8

"Novel roles for the mitotic kinase Nek7 in post-mitotic neurons"

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Abstract

In cycling cells, reorganization of the microtubule cytoskeleton is intimately related with cell cycle progression, both involving a great number of key regulators. Later on, it became known that some of these genes are also expressed in post-mitotic cells, with similar or alternative roles. During my PhD, I aimed to screen a short list of "mitotic/microtubule cytoskeleton-related genes" that are expressed in cultured mouse hippocampal neurons, by knocking down their expression through RNAi. Depletion of the mitotic kinase Nek7 in neurons at an early stage of differentiation (6DIV - days *in vitro*) generates a significant increase in axon length, while decreasing overall dendrite length and the number of dendrites per cell. These phenotypes can be rescued by co-expression of an shRNA-resistant mutant, and are mimicked by over- expression of an inactive kinase mutant.

In mitosis, Nek9 activates Nek7 and Nek6, and in their turn they phosphorylate the kinesin Eg5. Surprisingly, depletion of Nek9 in neurons does not generate the same phenotype as Nek7, suggesting that this pathway is not conserved. Nek6 KO mice neurons show an increase in axon length, but no effect on dendrites, indicating that in this context Nek6 and Nek7 might not be totally redundant. Eg5 is a known regulator of axon length, and an interaction between Nek7 and Eg5 in post-mitotic cells was confirmed by immunoprecipitation.

Nek7 protein levels are detected in the mouse brain during development, and are up-regulated as neurons differentiate *in vitro.* Nek7-depleted mature neurons (14DIV) have thinner and less dense dendritic spines (post-synaptic structures). Interestingly, these phenotypes were not observed for Nek6 or Nek9 depletion, suggesting a specific function for Nek7 at this stage. Recently published data showed that Nek7 regulates microtubule dynamics, which is intrinsically related with spine morphology. Overall, these results point to new functions for Nek7 in post-mitotic neurons, in the regulation of neuron morphology and possibly synaptic transmission.

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Talk 9

"Androgen receptor oligomerization in spinal bulbar muscular atrophy: a study at the residue level"

Giulio Chiesa[1], Bahareh Eftekharzadeh[1], Alessandro Piai[2], Jesus García[1], Isabella Felli[2] & Xavier Salvatella[1,3]

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Abstract

Spinal Bulbar Muscular Atrophy (SBMA), together with Huntington Disease, is a member of the polyglutamine (polyQ) expansion diseases family.Androgen Receptor (AR) is a nuclear receptor sensible to testosterone, which has an intrinsically disordered N-terminal domain that bears a polyQ tract. Aggregates of this polyQ-expanded protein have been observed in the motor neurons of SBMA patients. In vitro studies showed that aggregation of Androgen Receptor takes place only in presence of testosterone[1] and that the cleavage of the protein by caspase 3 is a crucial event for cytotoxicity [2]. In the polyQ protein field, an increasing body of evidence [3,4] supports the hypothesis that the aggregation of these proteins is controlled by regions flanking the polyQ tract, that can increase or decrease the rate of aggregation depending on their secondary structure.
We produced a recombinant cas-3 cleaved N-terminal fragment of AR with the polyQ region bearing different numbers of repeats and we developed a set of biophysical methods for characterizing the aggregation of this proteins in a reproducible and quantitative way.
With a sedimentation assay monitored by HPLC we observed that the number of repeats positively correlates with an increase in the aggregation rate and by Electron Microscopy and Atomic Force Microscopy we observed that the protein forms amorphous aggregates that reorganize in amyloid-like fibrils with time. Circular Dichroism data and NMR data indicate that the polyQ tract of AR with 25 repeats adopts a fully α-helical conformation, unlike other polyQ proteins already studied in literature [5,6]. Dynamic Light Scattering data demonstrated that the formation of early oligomers is not associated to the presence of a polyQ tract in AR. We therefore studied at the residue level the sequences involved in the various stages of the aggregation of the AR protein.
We found that the early oligomerization step is driven by interactions taking place in a region N-terminal to the polyQ (^{23}FQNLFSVREVI^{34}), as is shown by a time-resolved NMR experiment. We also identified a second region important for the aggregation in the polyLeu motif directly flanking the polyQ (^{54}LLLL^{58}), as mutations perturbing that sequence prevent the recombinant protein to evolve into insoluble aggregates.
These findings describe the mechamism of the aggregation of the Androgen Receptor protein and suggest possible ways to prevent the aggregation of this protein, delaying or preventing the onset of the disease in patients bearing a pathologic number of glutamine in this locus.

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Talk 10

"Mitochondrial stress in sporadic and familial form of ALS"

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Abstract

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder leading to progressive and selective loss of both upper and lower motor neurons. Sporadically occurring disease (sALS) affects the majority of patients (90 %) while the rest of cases is inherited and referred to as familial ALS (fALS). The clinical and pathological features of sporadic and familial forms of ALS in most cases are indistinguishable, which may suggest that they share similar pathogenic mechanism. Studies on animal and cellular models of ALS as well as the investigations of patients' tissues reveal changes in mitochondrial morphology, bioenergetics and calcium homeostasis. Understanding the relationship between mitochondrial malfunctions and progress of ALS may provide a useful tool for early diagnosis and potential pharmacological targets for treatment of the disease.

The presence of possible mitochondrial stress was verified by investigation of mitochondrial physiology in primary fibroblasts derived from patients diagnosed with sporadic and familial form of ALS and age matched control subjects. Because mitochondrial distribution in the cell is involved in neurodegenerative process we checked the level of proteins engaged in mitochondrial dynamics as well as the autophagy and apoptosis. The mitochondrial structure and organization were visualized by confocal microscopy.

We found decreased mitochondrial membrane potential and increased cytosolic [Ca^{2+}] in cells with both forms of ALS. The level of proteins involved in autophagy was higher in fibroblasts from patients with familial ALS suggesting the activation of this process. Control flux coefficient calculated on the basis of titration with specific respiratory inhibitor (sodium amytal) was higher in fibroblasts with sporadic form of ALS while maximal respiration rate on complex I substrates was slightly lowered which may suggest abnormalities in functioning of the complex. Organization of mitochondrial network in ALS cells as well as the profile of proteins involved in dynamics of this organelles was changed in comparison to control cells.

Talk 11

"Structural characterization of the DNA uptake machinery in gram-positive bacteria"

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Abstract

Horizontal Gene Transfer (HGT) [1] is an evolutive mechanism by which entire genes are transferred among bacterial cells, thus enabling an immediate adaptation to new environmental conditions. HGT has obvious implications for human health as it is used by pathogenic microorganisms – even among different species– during the spread of virulence factor and antibiotic resistance. Three main routes can be distinguished: conjugation (plasmid transfer through the direct interaction of two bacterial cells), transduction (bacteriophage-mediated DNA transfer) and transformation (uptake of naked DNA from the environment) [2] [3]. Although conjugation and transduction have been widely studied at the structural level, there is little knowledge about bacterial transformation. The term DNA Uptake Pump (DUP) refers to the translocating machinery used by bacteria to incorporate naked DNA from the environment to the cytoplasm during bacterial transformation.
The specific aim of this project is to determine the structure of the DNA uptake pump responsible for bacterial transformation in gram-positive bacteria using X-ray cystallography. This machinery is mainly formed by a DNA receptor, a transmembrane channel that mediates DNA translocation across the cytoplasmic membrane and an ATPase which pulls DNA into the cytoplasm at the expense of ATP.
We decided to adopt a high-throughput approach to achieve our goals. We designed 96 constructs from five different species with several solubility-enhancing fusion partners and affinity tags. The constructs can be divided into three groups: putative soluble constructs, fragments containing trans-membrane helices and full-length integral membrane proteins. We already have successfully cloned almost all the target genes and have tested them for soluble expression in E. coli. Large scale purification and preliminary crystallization trials are in progress for the soluble constructs.

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Talk 12

"The Drosophila histone H1 variant, dBigH1, regulates zygotic genome activation and plays an essential role in germline development"

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Abstract

Histone H1 is an essential chromatin component. Metazoans usually contain multiple stage- specific H1s. In particular, specific variants replace somatic H1s during early embryogenesis. In this regard, Drosophila was an exception because a single dH1 was identified that, starting at cellularization, is detected throughout development in somatic cells. Here we identify the embryonic H1 of Drosophila, dBigH1. dBigH1 is abundant before cellularization occurs, when somatic dH1 is absent and the zygotic genome is inactive. Upon cellularization, when the zygotic genome is progressively activated, dH1 replaces dBigH1 in the soma, but not in the primordial germ cells (PGC), that have a delayed zygotic genome activation (ZGA). In addition, a loss-of-function mutant shows premature ZGA. Mutant embryos die at cellularization, showing increased levels of RNApol II and zygotic transcripts, along with DNA damage and mitotic defects.
In adults, dBigH1 is abundant in ovaries and testis with a peculiar expression pattern: is present in the germ stem cells (GSC), is not present in the proliferating cells and reappears in the spermatocytes (males) and in the oocyte and nurse cells of the egg chamber (females). dBigH1 plays an essential role in germline development both in males and females as the loss of dBigH1 changes dramatically the expression of Bam, one of the key regulators of this process. In males, these changes produce spermatogonia accumulation, cell division problems and a decrease of spermatocyte differentiation which leads to fertility reduction.

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Talk 13

"Low-n Aβ oligomers: globular structure and well-defined cross-linked mimics"

Sílvia Vilaprinyó-Pascual,[1#] Rosa Pujol-Pina,[1#] Roberta Mazzucatto,[1] Annalisa Arcella,[2] Marina Gay,[3] Montserrat Serra-Batiste,[1] Marta Vilaseca,[3] Modesto Orozco,[2,4] & Natàlia Carulla[1*]

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Abstract

Amyloid-β (Aβ) dimers derived from the brains of Alzheimer´s disease (AD) patients are strongly associated with this pathology. Therefore, characterizing the structure of low-n Aβ oligomers—where n refers to the order of the oligomer—and preparing well-defined synthetic mimics of these oligomers is critical in the AD field. To address these needs, we first optimized the photo-induced cross-linking of unmodified proteins (PICUP) reaction to produce low-n cross-linked (CL) Aβ oligomers of defined order while avoiding secondary oxidation byproducts. This reaction offers the advantage that cross-links are defined by the intrinsic structure of the oligomers formed in solution. Moreover, cross-links are formed through the same mechanism by which reactive oxygen species (ROS) could lead to the production of cross-linked Aβ oligomers in the brain. Second, we compared the structural features of the CL oligomers with their corresponding non-covalent counterparts using ion mobility coupled to mass spectrometry (IM-MS). Using this strategy, we found that CL oligomers are excellent mimics of their non-covalent counterparts both in terms of the oligomer distribution obtained, ranging from dimers to tetramers, and of their structure. Third, we developed a method to isolate low-n CL oligomers of defined order. Finally, using IM-MS, circular dichroism (CD), and a proteomic strategy to identify the cross-link positions, together with atomistic molecular dynamics simulations, we determined that strikingly low-n Aβ oligomers have a globular structure. Given that we can prepare and isolate CL oligomers of defined order, our findings open up new avenues to study the role of specific low-n Aβ oligomers in AD as well as to use them as standards to define the chemical nature of brain derived SDS-resistant oligomers.

Talk 14

“One of the components of neuronal nuclear architecture: PML nuclear bodies”

Malgorzata Hanna Hall

Abstract

PML is a tumor-suppresor protein involved in the pathogenesis of promyelocytic leukemia. In non- neuronal cells, PML is a principal component of characteristic nuclear bodies. In the brain, PML has been implicated in the control of embryonic neurogenesis, and in certain physiological and pathological phenomena in the adult brain. Yet, the cellular and subcellular localization of the PML protein in the brain, including its presence in the nuclear bodies, has not been investigated comprehensively. Because the formation of PML bodies appears to be a key aspect in the function of the PML protein, we investigated the presence of these structures, and their anatomical distribution, throughout the adult mouse brain. We found that PML is broadly expressed across the gray matter, with the highest levels in the cerebral and cerebellar cortices. In the cerebral cortex PML is present exclusively in neurons, in which it forms well-defined nuclear inclusions containing SUMO-1, but not Daxx. At the ultrastructural level, the appearance of neuronal PML bodies differs from the classic one, i.e. the solitary structure with more or less distinctive capsule. Rather, neuronal PML bodies have the form of small PML protein aggregates located in the close vicinity of chromatin threads. The number, size and signal-intensity of neuronal PML bodies are dynamically influenced by immobilization stress and seizures. Our study indicates that PML bodies are broadly involved in activity-dependent nuclear phenomena in adult neurons.

Talk 15

"Structural bases for the interaction and stabilization of the human amino acid transporter LAT2 with its ancillary protein 4F2hc"

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Abstract

Heteromeric amino acid transporters (HATs) are the unique example known in all kingdoms of life of solute transporters composed of two subunits linked by a conserved disulphide bridge. In metazoans, the heavy subunit is responsible for the trafficking of the heterodimer to the plasma membrane, and the light subunit is the transporter. HATs are involved in human pathologies like aminoacidurias, tumor growth and invasion, viral infection and cocaine addiction. Beside this relevance the structural information concerning interactions between heavy and light subunits of HATs is very scarce. In this work transmission electron microscopy and single particle analysis of purified human 4F2hc/LAT2 heterodimers overexpressed in the yeast Pichia pastoris, together with docking analysis and crosslinking experiments reveal that the extracellular domain of 4F2hc interacts with LAT2 covering almost completely the extracellular face of the transporter. 4F2hc increases the stability of the light subunit LAT2 in detergent-solubilized Pichia membranes allowing functional reconstitution of the heterodimer into proteoliposomes. Moreover, the extracellular domain of 4F2hc suffices to stabilize solubilized LAT2. The interaction of 4F2hc with LAT2 gives first insights on the structural bases for light subunit recognition and stabilizing role of the ancillary protein in HATs.

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Talk 16

"RARRES3 suppresses breast cancer lung metastasis by regulating adhesion and differentiation"

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Abstract

Despite a recent decrease, breast cancer is one of the most common cancers in humans and will on average affect up to one in eight women in their lifetime in the United States and Europe. In Estrogen Receptor Negative breast cancer patients, metastatic relapse usually occurs in the lung and is responsible for the fatal outcome of the disease. Therefore, a better understanding of the biology of breast cancer lung metastasis is required. In particular, biomarkers to identify patients that are at risk of lung metastasis could open the avenue for new therapeutic opportunities.
Here we show that RARRES3 is a metastatic suppressor gene in breast cancer. Using the ER- MDA-MB-231 breast cancer cell line model and lung metastatic derivatives, we functionally validated that RARRES3 loss of expression confers a selective advantage for the colonization of the lung in vivo (xenografts and syngeneic models). RARRES3 silencing engages metastasis initiating capabilities by facilitating extravasation and adhesion of the tumor cells to the lung. Furthermore, RARRES3 phospholipase A1/A2 activity contributes to tumor cell differentiation, thereby blocking lung metastasis demonstrated in 3D organotypic cultures and by a re-initiation assay in vivo. Therefore, our results show that genes selected for metastasis contribute to the different steps of

this process and represent the random accumulation of traits that provide the necessary advantage for adaptation to the microenvironment of a different organ.
RARRES3 restrains the lung metastatic capacity of breast cancer cells and more important, RARRES3 levels in the primary tumor are clinically relevant as may predict risk of relapse. The contribution of RARRES3 to differentiation over self-renewal suggests that reduced RARRES3 expression could be also predictive of therapy-resistant tumors, identifying patients possibly requiring new therapies designed to target breast cancer-initiating cells. Therefore, screening for compounds that activate RARRES3 may contribute to the development of new differentiation -inducing strategies to target therapy-resistant breast tumors.
MAIN POINT: Functionally validate RARRES3 as a lung metastatic tumor suppressor gene in breast cancer in vitro, in vivo, and the most important, the clinical relevance in a human cohort. Therefore, we showed the potential importance of RARRES3 in therapy, i.e. screening for compounds that activate RARRES3 may contribute to the development of new differentiation -inducing strategies to target therapy-resistant breast tumors.

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Talk 17

"FROM BEE VENOM TO BLOOD-BRAIN BARRIER SHUTTLES:

apamin derivatives are capable of transporting antibodies across the barrier"

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Abstract

An ageing population and the growing prevalence of tumors are leading to an increase in the incidence of central nervous system (CNS) diseases. Unfortunately, most compounds intended for CNS treatment cannot reach their targets in therapeutically relevant amounts because they are unable to cross the blood-brain barrier (BBB).

One of the least invasive strategies to transport cargos into the brain parenchyma is using peptide vectors, also known as BBB-shuttles, that undergo transcytosis across brain-capillary endothelial cells.[1] However, the efficiency of existing shuttles is still low. This thesis focuses on the discovery of new protease-resistant peptides capable of carrying compounds across the BBB. In particular, we aimed to enhance the transport of antibodies against glioblastoma across the BBB to reach the invasive front of the tumor and reduce metastases and relapse.

In our quest for new shuttles, we turned our attention towards venoms because many of these molecules affect the CNS.[2] Among them, bee venom contains apamin, one of the few peptides that have been suggested to reach the brain without disrupting the BBB.

In this work, we demonstrated that the residues involved in toxicity are not relevant for transport in a cell-based model of the BBB, showing that non-toxic apamin derivatives were promising BBB-shuttle candidates.[3] We then produced a variety of more pharma-friendly analogs. All of them showed a similar permeability to that of the parent peptide and their half-life in serum was over 24h. Two of the new derivatives were conjugated to a variety of cargoes, ranging from fluorophores and small peptides to proteins and nanoparticles. All conjugates displayed remarkable permeability and the transport of large cargoes was enhanced.[4]

We then tested their capacity to transport monoclonal antibodies. We first explored a variety of conjugation techniques to control the number and the location of peptides linked to immunoglobulins. After characterizing the conjugates, we assessed the effect of these modifications on binding affinity by ELISA. We then assayed the compounds in the BBB cell-based model. Remarkably, these analogs enhanced the permeability of antibodies, one of them providing up to 7-fold increase. This analog will be assayed in an orthotopic glioblastoma murine model at the VHIO.

In conclusion, our results show that apamin-derived shuttles have the capacity to carry diverse cargoes across a cell-based model of the BBB. This breakthrough opens up a wide range of possible applications for these compounds, especially for the transport of monoclonal antibodies targeting CNS diseases.

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Poster Abstracts

Group 1

"Preliminary study of the role of DOR in mitophagy"

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Abstract

The accumulation of damaged mitochondria has been proposed as a key issue in aging and in the pathogenesis of many common age-related diseases, including Parkinson's disease (PD). Recent work suggests that two of the genes involved in familial forms of PD, PINK1 and Parkin, act in a common pathway regulating mitochondrial quality control. Upon mitochondrial depolarization, Parkin, an E3 ubiquitin ligase is recruited to dysfunctional mitochondria by PINK1, where ubiquitinates mitochondrial proteins for proteosomal degradation and promotes the engulfment of mitochondria by autophagosomes.
DOR is a nuclear protein that was recently showed to activate autophagy. Thus, we suggest that DOR might act as a marker to target mitochondria to autophagosomes. Our preliminary results showed that DOR is recruited to mitochondria in response to mitochondrial depolarization. Moreover, DOR slightly enhances the recruitment of LC3 to mitochondria. Furthermore, we found that DOR and Parkin co-localize, and when we carried out pull-down experiments these proteins co-immunoprecipitate.

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"The impact of the Circadian Clock deregulation during Aging of Epidermal Stem cells"

Peixoto FO, Solanas G, Benitah SA

Abstract

Our lab has shown that the clock machinery not only provides epidermal

stem cells (epSCs) with temporal functional cues along the day, but also that the desynchronization of the stem cell clock in vivo disrupts tissue homeostasis, accelerates tissue aging, and predisposes the tissue to tumorigenesis.

Our aim is to understand how and why the epSC clock targets change in epSCs with age. We have now isolated epSCs at 6 times per day from 3-months and 24-months old mice, and performed transcriptomics analysis. We have observed that 80% of the circadian oscillating genes in young epSC ceases to be circadian in aged epSC. Strikingly, many genes become newly circadian in aged epSC. This suggests a change in the clock-controlled genes during physiological aging. We also want to understand if such changes are due to intrinsic or extrinsic factors. To rule out the importance of intrinsic changes, we have optimized the culture of epSCs from young and old mice. Regarding systemic cues, we want to understand the role of metabolism on the epSC clock. For this reason we are performing an experiment with young and aged animals subjected to caloric restriction and their counterpart controls.

"EXD2: a conserved mitochondrial exonuclease that influences mtDNA levels and aging"

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Abstract

Nucleases are required for a number of important cellular functions including DNA replication, DNA repair and transcriptional and translational regulation. The putative 3'-5' exonuclease domain containing protein 2 (EXD2) shares significant homology to the exonuclease domain of the WRN gene that is mutated in Werner's syndrome (WS) progeria. WRN prevents telomere loss and WS patients are predisposed to a wide variety of cancers. In addition, EXD2 was implicated in DNA crosslink repair in a large-scale siRNA screen. We sought to determine the cellular roles of EXD2 and determine if it played a role in maintaining genomic stability. We found that EXD2 localizes to the mitochondria through an N-terminal mitochondrial targeting sequence that is both necessary and sufficient for mitochondrial localization. Using a modified ChIP procedure, we demonstrated that EXD2 interacts with the mtDNA and identified residues critical for the interaction. Depletion of EXD2 by siRNA leads to increased mtDNA levels and extensive metabolic alterations but does not impair mtDNA replication. In order to examine the roles of EXD2 in vivo, we obtained a strain of Drosophila melanogaster with a transposon insertion in the orthologous gene, CG6744 (DmEXD2). DmEXD2 deficiency leads to an extended pupation period and a significant increase in female lifespan that is independent of caloric restriction. The extended lifespan is accompanied by decreased fecundity and the depletion of the germ stem cells (GSCs). We also found that expression of several insulin-related genes and the ecdysone receptor, both of which have been associated with GSC maintenance and lifespan, were reduced in Females lacking DmEXD2. In addition, these flies were more sensitive to oxidative stress,

including hypoxia and antioxidant diets. We hypothesize that EXD2 activity is regulated by oxidative stress in order to fine tune mitochondrial metabolism. Current studies are focusing the identification of its precise substrates in order to understand the mechanism of its influence on development and aging. Current data on our ongoing characterization of mammalian and Drosophila EXD2 will be presented.

Group 2

"MicroRNAs expression profile of the dentate gyrus in a rat model of temporal lobe epilepsy"

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Abstract

MicroRNAs are noncoding RNAs acting by degradation or destabilization of target mRNAs. Recent studies have suggested the contribution of miRNAs in neurodegenerative diseases, however its role in epilepsy remains still unknown. The aim of the study was to investigate changes in expression level of miRNAs in the dentate gyrus of epileptic animals. Epilepsy was induced in adult Sprague-Dawley rats by status epilepticus evoked by electrical stimulation of the left lateral nucleus of the amygdala (100-ms train of 1-ms biphasic square-wave pulses delivered at 60 Hz, every 0.5 s for 30 min). To determine the frequency of spontaneous seizures animals were constantly monitored with video EEG. Brain samples were collected at 7, 14, 30, 90 days after stimulation (n=5). Total RNA enriched in microRNA fraction was isolated from the left dentate gyrus of epileptic and sham operated animals with miRNeasy mini kit (QIAGEN) and profiled using miRCURY LNATM microRNA Array 7th (EXIQON) with the miRBASE version 19.0.
Analysis of miRNAs showed significant changes in expression of 66 miRNAs ($p<0.05$) in stimulated animals as compared to sham operated controls. Nine miRNAs were up-regulated, while 57 miRNAs were down-regulated. In silico analysis of miRNAs expression profile revealed potential genes targets for these miRNAs and hierarchical clustering analysis discriminated the epileptic animals from the controls. This data suggest involvement of miRNAs in epileptogenesis or epilepsy.

"CPEB4 is requered to mantain genomic stability"

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Translational control of gene expression has acquired increasing relevance as a mechanism involved in many physiological processes in eukaryotes, such as the regulation of embryonic development, cell differentiation and proliferation, but also during pathological events as cancer. One important mechanism of translational control involves cytoplasmic changes in the poly (A) tail length of the mRNAs through the CPEBs (Cytoplasmic Polyadenilation Element Binding proteins). CPEB4 is the least characterized member of the family of four RNA-binding proteins. To further study the role of CPEB4 in mammals, we have generated a tamoxifen-inducible CPEB4 KO mouse model. Depletion of CPEB4 in adult mice caused skin hyperpigmentation and loss of epidermal stratification. Primary culture of CPEB4-null keratinocytes revealed diminished growth capacity due to dysfunctional cell division. Accumulation of DNA damage was detected in KO keratinocytes along with the presence of micronuclei, DNA bridges and chromosomal fusions. All these alterations seemed to originate from defects in the DNA replication process such as lower fork progression and inefficient fork restart that drived the accumulation of unstable ssDNA. We performed RNA-immunoprecipitation sequencing to discover the mRNAs regulated by CPEB4 in this context and found that CPEB4 regulates a network of cancer related pathways that includes genes such as p53, myc and Kras.

"The role of dermal fibroblasts in skin aging and cancer"

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Abstract

As epidermal stem cells age they gradually fail to regenerate the skin in response to physiological cell turnover and injury. Moreover, aged stem cells are more prone to tumorigenic transformation. These stem cell defects are partly caused by the accumulation of cellular damage within the stem cells themselves, however, as shown in other tissues, changes in the stem cell niche during aging may also contribute. In order to gain insight into the role of the stem cell niche in skin aging, I am investigating age-related changes in dermal fibroblasts and how these changes may affect the behavior of epidermal stem cells in the context of aging and cancer.

Group 3

"Breast Cancer Genes PSMC3IP and EPSTI1 Play a Role in Apoptosis Regulation"

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Abstract

Breast cancer is the second most frequently diagnosed cancer in women, with one million of new cases every year worldwide. The balance between proliferation and apoptosis is crucial in determining the overall growth or regression of the tumor in response to chemotherapy, radiotherapy and hormonal treatments. Thus, it is possible to delineate the biology of individual tumors at the molecular and biochemical level by examining apoptosis and its control and regulation, and to exploit these to clinical advantage. To this end, we have selected genes potentially involved in apoptosis based on a breast cancer protein network analysis carried out in our group (Arroyo et al. submitted). We have induced or inhibited our candidate genes in different human breast cancer cell lines by means of overexpression or gene silencing experiments, respectively, and subsequently applied apoptotic assays to evaluate the role of these genes in apoptosis in the context of breast cancer. Our preliminary findings suggest a new antiapoptotic role for two breast cancer associated genes. Further experiments are required to clearly elucidate the potential therapeutical applications of these genes in breast cancer treatments.

"The insulator protein CTCF regulates Drosophila steroidogenesis"

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Abstract

Proper development of multicellular organisms requires accurate timing of specific developmental programs of gene expression, such as signaling systems that respond to nutritional and environmental allowing transitions in morphology (molting and metamorphosis in insects) which occur at regularly defined intervals and depend on pulses of the steroid hormone ecdysone. The prothoracic gland (PG) is the tissue responsible for the synthesis of ecdysone. The process is initiated by PTTH, which leads to increased transcription of ecdysone biosynthetic enzymes encoded by the Halloween gene family.
The insulator protein CTCF play an essential role in regulation of chromatin organization and gene expression during development.
Our results indicate that:
CTCF controls the timing of larval development: developmental timing in

CTCF mutants was prolonged by two days.
Loss of CTCF in ecdysone-producing cells impairs Halloween gene transcriptional activation: we knocked down CTCF specifically in the PG and transcript levels of some Halloween genes, spok, dib and sad showed only small increases during the prolonged third larval instar, but it does not interphere with PTTH signaling.
CTCF expression in the PG is required for proper timing of ecdysone-signaling gene expression: we measured the transcript levels of ecdysone-responsive genes, and they are delayed respect to controls, in the similar way of pupariation. The production of ecdysone, not the response to the hormone, is the source of delay in CTCF knockdown animals.
Developmental timing depends on CTCF-mediated cholesterol homeostasis and ecdysone synthesis: we fed either 20E or cholesterol. Both recover partially, but only efficiently rescue developmental delay is obtained by feeding with both, suggesting that CTCF is needed not only to synthesize ecdysone from cholesterol but also to mediate cholesterol homeostasis in PG cells.

CTCF is required for lipid homeostasis in the PG: lipid droplets in PG cells seen at the onset of pupariation is reduce in comparison to control in CTCF due to developmental delay. These data along with results reported above showing partial rescue in cholesterol-fed CTCF-knockdown larvae indicate a role of CTCF in sterol homeostasis in the PG.

References…

"Differential effects of the Tousled like kinases 1 and 2 on mammalian development"

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Abstract

Tousled like kinases (TLK1, TLK2) are serine/threonine kinases linked to ongoing DNA replication, DNA damage sensitivity and chromatin deposition. Both proteins have been demonstrated to interact with the histone H3/H4 chaperone ASF1, which plays essential roles in DNA replication and repair, transcriptional regulation and chromatin silencing. In human cells, TLKs phosphorylate ASF1 and are inactivated by the checkpoint kinase CHK1 following DNA damage, linking chromatin assembly and the DNA damage response (1). Downregulation of Tousled like kinase activity compromises the regulation of histone chaperone ASF1, causes chromosomal instability, DNA damage sensitivity and impaired development in lower organisms. To understand the relative functions of mammalian TLK1 and TLK2, we have generated mouse models to study TLK function in vivo and at the cellular level, and established several assays to study TLK kinase activity. We find that TLK1 is dispensable for mammalian development, chromosome stability and DNA damage sensitivity. In contrast, Tlk2 null embryos are embryonic lethal

due to placental failure. Surprisingly, we find that despite similar levels of mRNA, little TLK1 protein is expressed in the placenta. The conditional bypass of this placental defect allows the generation of apparently normal TLK2 deficient animals, suggesting that they play redundant roles in most tissues. In addition, we have identified key regulatory sites in the TLK2 kinase domain activation loop that shed light on its molecular regulation and will allow the generation of phospho-specific antibodies to track its activation state. This work has provided the first evidence for a role of TLK activity in mammalian development and we will present ongoing genetic and biochemical characterization of these proteins.

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Group 4

"CEP63 deficiency promotes p53 dependent microcephaly and reveals a role for the centrosome in meiotic recombination"

Berta Terré Torras

Abstract

Significant overlap in the pathology of human diseases caused by defects in the DNA damage response (DDR) or centrosome homeostasis suggests that they may have similar underlying mechanisms. Seckel syndrome is caused by deficiency in the DDR proteins ATR or CTIP or centrosomal proteins, such as PCNT or CenpJ. Seckel patients present with severe dwarfism, microcephaly, mental retardation and metabolic abnormalities. In addition, a number of Seckel mouse models have been shown to have defects in fertility. We have been investigating the functions of Cep63, a centrosomal protein that was identified as an ATR substrate and has recently been implicated in Seckel syndrome, as it provides a potential link between the DDR and the centrosome. To determine the consequences of Cep63 deficiency, we have generated animals lacking expression of all known isoforms of Cep63. We find that the DNA damage response is largely intact, however Cep63 deficient cells display abnormal centrosome configurations and chromosomal instability. Animals

lacking Cep63 recapitulate the mild Seckel syndrome reported in human patients as they are runted and develop microcephaly that is p53 dependent. In addition, we have found that Cep63 deficient male mice are infertile and have severe defects in testicular development. In contrast, Cep63 deficient females are fertile with normal litter sizes and Mendelian ratios. The drastic decrease in Cep63 deficient testes size is accompanied by an increase in apoptotic tubules that is independent of p53. Moreover, Cep63 deficient mice do not produce any mature sperm, although some elongated spermatids are detected in testes sections. Further analysis revealed that Cep63 deficiency provokes defects in the progression through meiotic prophase I, with enrichment of cells in leptonema and zygonema. Cep63 deficient spermatocytes also progress poorly through pachynema with only few cells reaching diplotene stage. To address the effects of Cep63 deficiency on meiotic recombination, we have analyzed the induction of double strand breaks (DSB's) by scoring RAD51 and DMC1 foci, cytological markers of DSB's. Our analysis suggests that the timing of breaks in Cep63 deficient mice is normal. However, they have increased numbers of both markers per nuclei from late leptonema to late zygonema. Moreover, the total portion of Cep63 pachytene cells with crossovers, as quantified by MLH1 foci, was significantly diminished, consistent with problems progressing normally through pachynema. We reason that these meiotic aberrations in Cep63 deficient animals may be the result of centrosome-dependent microtubule defects that impair chromosome homology search and bouquet formation. We propose that in addition to being a model system for studying microcephaly, Cep63 animals also provide a model for understanding the functions of the centrosome in male fertility. Current results from the ongoing characterization of these animals will be presented.

"Determinants of Eg5 centrosomal accumulation in prophase"

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Abstract

Accumulation of the microtubule plus end-directed kinesin Eg5 at centrosomes during the G2/M transition is essential for motor-directed centrosome separation during prophase and thus normal spindle assembly. Downstream of CDK1 and Plk1, the NIMA-family kinases Nek9, Nek6 and Nek7 form a signaling module that regulates this process through Nek6/7 phosphorylation of Eg5 at Ser1033. Interference with Eg5[Ser1033] phosphorylation prevents centrosomal accumulation of the kinesin and thus prophase centrosome separation as well as speedy and accurate mitosis. The mechanism of Eg5 accumulation around centrosomes at prophase is still unclear. We have studied the molecular determinants of this process, with the aim of understanding how Eg5 is accumulated at a region rich in microtubule minus ends and how the kinesin contributes to motor-directed centrosome separation before nuclear envelope breakdown.

"CPEB proteins during cell division"

Rosa Pascual[1] and Raúl Méndez[1].

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Abstact

Eukaryotic transcripts generally contain a m7Gppp group at the 5' untranslated region (5' cap) and a tail of 200-500 nucleotides of adenine residues (poly(A)tail) at the 3' untranslated region (3' UTR). One of the key mechanisms accounting for translational control of the mRNA is the regulation of the length of the poly(A) tail. Longer poly(A) tails are associated to more efficient translation and higher stability of mRNA. In fact, 20% of human transcripts are predicted to harbor cis-acting elements on their 3'UTR named Cytoplasmic Polyadenylation Elements (CPE), which are recognized by CPE-Binding (CPEB) proteins that can either promote or repress polyadenylation of mRNA (Belloc and Mendez, 2008; Fernandez-Miranda and Mendez, 2012; Pique et al., 2008). In vertebrates, the CPEB family is composed of four RNA-binding proteins (CPEB1-4), where CPEB1 is the most distant one from a structural and evolutionary point of view (Fernandez-Miranda and Mendez, 2012). Yet CPEB1 is the best well-studied member of this family. CPEB1 was discovered in Xenopus laevis oocytes where it is responsible of the meiotic progression by activation of maternal mRNAs through elongation of their Poly(A) tail upon progesterone induction (Hake and Richter, 1994). Lately, CPEB1 and CPEB4 have been shown to act sequentially for a proper mitotic cell-cycle progression (Novoa et al., 2010).

Previous data from our lab support the localization of CPEB1 in the spindle and the importance of CPEB1 for local translation at spindle poles of CPE-containing mRNAs. Interestingly, we observed that CPEB4 is localized at the spindle midzone, pointing to the interplay between CPEB1 and CPEB4 in the spindle. This fact suggests that CPEB1 and CEPB4 could act sequentially controlling local translation during cell division. I aim to study the role of CPEB4 in both symmetric and asymmetric cell division (ACD). As a model for polarity and ACD, I cultured mammary epithelial cells from both WT and CPEB4 KO mice into basement membrane matrix, where they form 3D acini (so-called mammospheres). We found that CPEB4 might have a role in spindle positioning, therefore determining the plane of division and affecting fate of the daughter cells.

Group 5

"In vivo model of dormancy in ER positive breast cancer to bone metastasis."

Sylwia Gawrzak [1], Marc Guiu [1], Jelena Urosevic [1], Esther Fernandez [1], Milica Pavlovic[1], Roger R Gomis [1]

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Abstract

Breast cancer is the most frequently diagnosed cancer and remains the second leading cause of death among women in Europe and United States. Majority of cases of patient death are caused by metastatic relapse. In the ER positive breast cancer, tumors are characterized by the late appearance of metastasis, years or decades after primary tumor resection. In this setting metastatic dormancy plays a relevant role. The identification of mechanisms that allow dormant metastases to become active is essential for understanding the biology of ER positive breast cancer metastatic latency and has putative implications for clinical practice. Therefore, we aim to establish xenograft model of dormancy in ER positive breast cancer to bone metastasis.

"Role of DOR/Tp53inp2 in the control of cellular proteostasis"

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Abstract

Protein homeostasis, or proteostasis, is a key mechanism by which cells rapidly respond to their environment to maintain cellular proteins in a state that allows optimum biological activity. To ensure dynamic protein turnover, eukaryotic cells have many complex pathways to regulate protein synthesis and degradation. Two major pathways degrade most cellular proteins: the ubiquitin-proteasome system (UPS) and autophagy. The nuclear cofactor DOR was identified originally as a protein expressed in PML of nuclear bodies [1]. However, in response to cellular stress, DOR exits the nucleus, localizes to early autophagosomes and regulates autophagy [2]. Moreover, recent data from our laboratory demonstrated that DOR promotes muscle wasting by the activation of basal autophagy in skeletal muscle [3].
In this project, we have analyzed the role of DOR in the regulation of the other degradation pathway, namely the UPS. Our results indicate that DOR is a negative regulator of the proteasome activity in C2C12 cells and mice skeletal muscle. Our data suggest that this upregulation of the proteasome activity in DOR-deficient cells and DOR knock out mice could be regulated by an induction of PSMD11, the subunit of the 19S regulatory particle involved in proteasome assembly.

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The role of the Colorectal Cancer Stem Cells (CRC-SCs) gene program in tumor behavior. "

Clara Morral

Abstract

Colorectal cancer (CRC) is the second cause of death by cancer. Our laboratory has developed an approach to isolate the Colorectal Cancer Stem cells (CRC-SCs) by using the tyrosine kinase receptor EphB2 gradient expression, which is also maintained in normal intestinal epithelium1. This specific cell population expresses high levels of the EphB2 receptor as well as elevated levels of intestinal stem cells specific genes like Lgr5. Moreover, it has been demonstrated that these EphB2- high cells display robust tumor-initiating capacity in immunodeficient mice as well as long-term self- renewal potential2.

Group 6

Elisa Montagni

Abstract

The small intestinal epithelium is the most rapidly self-renewing tissue of mammals. Proliferative cells are confined to crypts, while differentiated cell types predominantly occupy the villi. At the base of the crypts, a long- lived pool of cycling intestinal stem cells (ISCs) has been identified and profiled by using different markers. Mouse and human ISC signatures generated in our and other laboratories show the SPARC-related modular calcium-binding protein 2 (SMOC2) as one of the most consistent genes. Moreover, our gene expression profiles of several human colorectal cancer (CRC) samples show that SMOC2 is consistently enriched in the sorted ISC-like tumor cells. My project aims to the functional characterization of such a

novel gene SMOC2 in stemness and tumor initiation and progression.

"Molecular basis of p38 MAPK signaling"

Nuria Gutierrez

Abstract

p38α MAP Kinase (MAPK14) regulation involves interaction with other signaling proteins such as MAPK scaffolding proteins or phosphatases and to perform specific functions should phosphorylate downstream targets. Some components of these signaling pathways are well established, but we still poorly understand how the system is regulated in many cases.. Moreover, it is still unknown how the interaction of p38α with substrates and other molecules is regulated. In particular, it remains unclear which domains of p38α are implicated in the interaction with different molecules. Besides interacting with different proteins, p38α phosphorylates different substrates including transcription factors that regulate gene expression and amplify the signal. Several studies have described genes that are regulated p38α, but more comprehensive studies are required for the full identification of p38α regulated genes. Our goal is to try to identify new protein partners of p38α, news genes regulated for p38 MAPK signaling as well as the physiological role of different structural domains of p38α to better understand the properties and regulation of this signaling pathway.

"2-deoxyribose-induced apoptosis regulation distinguishes lymphoblasts of sporadic and familial Alzheimer's disease patients."

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Abstract

Objectives: We previously reported that control of cell cycle distinguishes lymphoblasts from sporadic and familial Alzheimer's disease patients (SAD and FAD). We found significantly increased basal p21 levels in SAD cells compared with FAD lymphoblasts. Since it is known that p21, besides controlling cell cycle, can regulate apoptosis, we checked whether p21 levels play a role in the cellular response of FAD and SAD cells to oxidative stress evoked by 2d-ribose (2dRib).

Methods: Cell viability after 2dRib and pifitrin (PFT–a) treatment were measured using MTT assay, mRNA levels were evaluated using real-time PCR and protein levels by immunoblotting. p21 levels in nuclear and cytosolic fractions were visualized using confocal laser scanning microscopy.

Results: FAD lymphocytes were more resistant to 2dRib-induced cell death

than control or SAD cells. In response to 2dRib FAD cells showed significantly increased p21 mRNA and protein levels and preferentially cytoplasmic location of p21 as compared to SAD cells. Transcriptional activation of p21 was shown to be dependent on p53, as it can be blocked by PFT-a.

Conclusions: The increase in p21 transcription in FAD lymphoblasts and its cytoplasmic localization confer these cells a survival advantage, since PFT-a sensitized FAD cells to 2dRib-induced apoptosis. Thus, as some cellular mechanisms seem to be different in FAD and SAD cells, our data suggest a possibility for differential diagnosis of FAD and SAD based on p21 and p53, and individualized therapeutic approach for SAD and FAD.

This research was supported by the Polish National Science Centre grant NN401 596840 to UW, the JPND grant 2/BIOMARKAPD/JPND/2012 to JK and UW, Rem Actu Tetigisti (RAT) 2014 scholarship to JW, and SAF2011-28603 (Spanish Ministry of Innovation and Competitivy) and Fundación Ramón Areces to AMR

Group 7

"Understanding the nature of the Nef requirement for HIV-1 infectivity"

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Abstract

Nef is an HIV -1 accessory protein with a fundamental role for virus replication in vivo and for the development of AIDS. Among its several activities, Nef is essential for maintaining HIV-1 maximal infectivity. In cell culture, such

activity can account for as much as 98% of the HIV-1 virion infectivity and requires Nef to be expressed in virus producing cells rather than in target cells. However, the cause of the defective infectivity of Nef-negative HIV-1 and the mechanism by which Nef is able to restore such a defect remain elusive.

We analyzed the ability of Nef to enhance HIV infectivity produced in more than 50 cell lines of different histological origin. We found that the requirement of Nef is highly variable. We identified a group of cell lines (Nef-responsive) in which the lentiviral protein is most required (10-50 fold) and a group of cell lines in which Nef is not at all or only weakly required (Nef-unresponsive, max 2-3 fold). All Nef-responsive cell lines belong to the lymphoid lineage, in line with the lymphotropic nature of HIV, while most cell lines belonging to the Nef-unresponsive group include mostly non-lymphoid cells. However, we indentified two Nef-unresponsive cell lines of lymphoid origin, which provide useful tools to investigate the nature of the Nef requirement. To study the dominance of the Nef requirement for infectivity, we have established a heterokaryon assay which allows HIV-1 production only upon fusion of producer cells expressing complementary parts of the virus. Data suggest that the requirement for infectivity is due to a restrictive cellular activity. We are now in the process of analyzing and comparing transcriptomes from cell lines with opposite Nef requirement in order to identify the cellular factor(s) counteracted by Nef.

“The role of the ãTuRC subunit GCP8 in cytoskeleton organization”

Sabine Klischies, Rosa María Ramírez, Artur Ezquerra and Jens Lüders

Abstract

Recently mass spectrometry of affinity-purified γTuRCs identified a novel subunit named MOZART2/GCP8. Our lab has shown that GCP8 is not required for γTuRC assembly but plays a role in γTuRC recruitment and microtubule nucleation at interphase centrosomes.

Surprisingly, RNAi-mediated depletion of GCP8 has no impact on mitotic progression, even though mitotic spindle assembly is defective in cells depleted of any of the other core subunits. Depletion of GCP8 in U2OS cells stably expressing EB1-GFP caused a profound increase in the growth speed of microtubules, affecting microtubule dynamics. The increased polymerization rate was not an indirect effect of globally impaired nucleation (and the resulting increase in the concentration of free tubulin) since global inhibition of nucleation (by depletion of the essential structural γTuRC subunit GCP3 or GCP6) caused only moderately increased polymerization rates. In addition we observed an increase in acetylated α-tubulin in the same cells, resulting in more stable but still more dynamic MTs.

Furthermore we identified GCP2 as the only binding partner for GCP8 at the γTuRC. By expressing deletion mutants for GCP2 and GCP8 we determined that the N-terminus non-conserved domain of the GCP2 and the N-terminus conserved region of GCP8 are the binding regions. Apart from GCP2 being the binding partner for GCP8, we also observed a co-depletion for GCP8 when depleting GCP2.

Considering that our published data demonstrates that GCP8 depletion does not disrupt γTuRC assembly/stability, we conclude that GCP8 has a specific role in microtubule

plus end polymerization acting through the γTuRC.

"^{18}F-FDG PET/CT imaging to monitor the anti-inflammatory effect of liposome-encapsulated Prednisolon Phosphate in experimental rheumatoid arthritis"

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Abstract

Aim:
Rheumatoid arthritis is a chronic autoimmune disorder involving joint inflammation and cartilage destruction. Treatment often includes systemic or intra-articular administration of corticoids. However, high and frequent dosing is required due to poor accumulation at target sites and rapid elimination of the drug. It has been shown that liposomal encapsulation of corticoids leads to enhanced joint targeting and anti- inflammatory effects. Early and non-invasive assessment of responsiveness would be beneficial to further improve treatment. Therefore, we aim to monitor the therapeutic effects of liposome-encapsulated prednisolon phosphate (PLP) in murine antigen- induced arthritis (AIA) using 18F-FDG PET/CT.

Methods:
A standardized preclinical AIA model, in which mono-articular arthritis was induced in male C57Bl6/J mice, was used. At 0, 3, 7 and 12 days after arthritis induction, inflamed joints were macroscopically scored (0 = unaffected, 4 = immobile) and 18F-FDG PET/CT images were acquired to monitor the course of the disease. After scanning, mice were dissected to investigate the biodistribution. To monitor therapeutic effects of liposome-encapsulated PLP, mice were treated with a single i.v. injection of PLP containing liposomes (10 mg/kg) or empty liposomes at day 3 after arthritis induction. 18F-FDG accumulation in inflamed joints (right) and unaffected joints (left) was quantified. Right-to-left (R/L) uptake ratios of the mean 18F- FDG uptake were determined.

Results:
Development of arthritis and therapeutic effects of liposome-encapsulated PLP was visualized using 18F-FDG PET/CT. 18F-FDG accumulation in inflamed joints increased significantly with progression of arthritis, with a maximum uptake at day 7 (day 0: 2.5 ± 0.9 %ID/g, day 7: 4.4 ± 0.4 %ID/g, $p = 0.02$; score day 0: 0 ± 0, day 7: 2 ± 1, $p = 0.002$), while no changes were observed in unaffected paws. After treatment with liposome-encapsulated PLP (10 mg/kg), joint swelling was suppressed (score: 0 ± 0, all time points). In line with that, 18F-FDG accumulation did not change over time (R/L ratios: 1.1 ± 0.1, 1.0 ± 0.1, 1.0 ± 0.1, 1.0 ± 0.1, days 0, 3, 7 and 12, respectively, $p = 0.35$). In mice that received empty liposomes, R/L ratiosincreased up to day 7 (R/L ratios: 1.0 ± 0.1, 1.4 ± 0.2, 1.4 ± 0.1, 1.1 ± 0.2, days 0, 3, 7 and 12, respectively, $p = 0.02$).

Conclusion:
18F-FDG accumulated in inflamed knee joints and reflected inflammatory activity. 18F- FDG PET/CT could be used to visualize the progression of AIA and to monitor the anti- inflammatory

effects of liposome-encapsulated PLP in arthritis.

Group 8

"Total chemical synthesis of D-vascular endothelial growth factor"

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Abstract

Vascular endothelial growth factor (VEGF) is a predominant regulator of tumor angiogenesis and its over-expression stimulates the generation of new blood vessels, and thus accelerates tumor growth. The development of improved antagonist of VEGF, which prevents the interaction of VEGF with its receptors, is an important current objective in medicinal chemistry. With the mirror image phage display methodology, new D-peptide ligands of VEGF can be obtained, which would be resistant to proteolytic digestion. The first step of this methodology involves the chemical synthesis of the mirror image form of the natural protein.
In this poster, we will explain the different methodologies for obtaining peptides and proteins applied to the synthesis of D-VEGF. Although, the stepwise synthesis of long peptidic sequences (102 amino acids) presents many challenges and low yields, it can be applied in the synthesis of VEGF. As an alternative, the combination of solid phase peptide synthesis (SPPS) and native chemical ligation (NCL) has been applied for the synthesis of many proteins, including VEGF [1-3]. In this procedure, the VEGF sequence was divided in several fragments for their separate synthesis. They contained a cysteine at the N-terminus and were functionalized with a thioester at the C-terminus to allow the NCL between two fragments. Here we will describe the best methodology and the synthetic challenges for the synthesis of this type of peptides. Also, we will describe how D-VEGF is obtained through the ligation of the several fragments in one-pot native chemical ligation. Finally, the covalent homodimeric protein is obtained with the subsequent folding/disulfide bond formation.
In conclusion, we describe and compare different methodologies for the synthesis of a challenging protein with a high therapeutic interest. The chemical accessibility of D-VEGF will make possible the modification of some positions for non-natural amino acids to be applied in the search of new antagonists of VEGF.

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"Development of Synthetic Strategies for Lasso Peptides with Anticancer Activity"

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Abstract

Lasso peptides are a structurally unique class of bioactive peptides characterized by a knotted arrangement, where the C-terminus threads through an N-terminal macrolactam ring.1 These ribosomally assembled peptides consist of 16–21 amino acids and share this N-terminal eight/nine-residue macrolactam ring where the terminal linear tail is trapped by steric hindrance of bulky side chains. They present a range of biological activities: antibacterial agents, inhibitors of HIV replication and selective antagonists of the endothelin type B receptor. They are classified in three subgroups, being class I and class II lasso peptides the most frequently encountered. Class I presents two disulfide bridges whereas Class II does not hold any. Some examples of each class are shown below:

Class I

MS-271 CLVCSCNDFAGCGYAIVCFW

RP71955 CLGIGSCNDFAGCGYAVVCFW

Class II

Capistruin GTPGFQTPDARVISRFGFN

Microcin J25 GGAGHVPEYFVGIGTPISFYG

Recently, a new lasso peptide, Sungsanpin,2 was isolated from Streptomyces strain collected off the coast of Sungsanpo on Jeju Island, Korea. Sungsanpin showed cytotoxicity in a cell invasion in a human non-small-cell lung cancer cell line A549.

Sungsanpin GFGSKPIDSFGLSWL

To date no synthesis is available to access lasso peptides due to the difficulty in building and maintaining the threaded lasso structure. The ability to generate lasso peptides synthetically would be a significant achievement and would also open the door to the production of lasso peptide analogs with unnatural amino acids or other non-proteinogenic building blocks. To design these small size and constrained structures would be very important from a therapeutic point of view.

"Synthesis of polypeptides and proteins using an improved cysteine-free native chemical ligation approach"

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Abstract

Total synthesis of large polypeptide and protein molecules remains one of the main today's scientific challenges despite the breakthroughs that were achieved with the development of solid phase peptide synthesis (SPPS) and native chemical ligation (NCL). The main drawback of SPPS is that can yield polypeptides of maximum ~30-50 amino acids in an efficient manner while in the case of NCL technique, the requirement of the Cys residue at the ligation junction limits its application. However, recent advances in the field of peptide synthesis opened the possibility to, through direct aminolysis, ligate two different peptides by using, theoretically, any amino acids pairs at the ligation junction.
We found that the addition of HOBt to the reaction mixture increases the conversion and rate of ligation, in some cases by more than 50%. Moreover, we were able to ligate phosphorylated peptides, which opens up the possibility of preparing modified protein domains by combination of proteins expressed in bacteria with peptides containing modified amino acids at specific positions, using cysteine-free approach.

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Group 9

"A comparative analysis of Multisubunit Tethering Complexes *in vivo*"

Irene Pazos

Abstract

Multisubunit Tethering Complexes (MTCs) form a group of 9 protein assemblies essential for vesicle trafficking. 42 different proteins organize to generate these complexes that are conserved from yeast to human. 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. This light microscopy method allow us to characterize MTCs from a functional and structural point of view in the yeast *Saccharomyces cerevisiae*. I will present our work to study the network of interactions among all the MTCs subunits, new components that associate transiently with MTCs (and their biological role), and the specificity cargo profile of this machinery.

"

SMemO: A Stabilized Aß42 Membrane Oligomer"

Montserrat Serra-Batiste[1], Martí Ninot-Pedrosa[1], Mariam Bayoumi[2], Margarida Gairí[3], Marta Vilaseca[4], Giovanni Maglia[2], Natàlia Carulla[1]

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Abstract

Amyloid beta (Aß) oligomers, intermediate species into the Aß aggregation process, are considered the pathogenic form of this protein in Alzheimer's disease (AD) (1). However, due to the high heterogeneity and transient character of these species, it is being very difficult to isolate and characterize them.
The aim of our project is to prepare biologically relevant stable Aß oligomers to be able to fully characterize them. Aß is obtained from the sequential cleavage of a membrane protein, the amyloid precursor protein, APP. Moreover, it is also described that Aß oligomers exert their neuron damage at the membrane (2). Therefore, we envisioned that a natural way of stabilizing them could be by studying them in a membrane environment.
We have studied two Aß variants, Aß42 and Aß40, which present two very different roles in AD. While Aß42 is the most strongly linked to AD, Aß40 is the most abundant Aß form. By screening different conditions, including several membrane environments, incubation times and temperatures, we were able to obtain stable, homogeneous and well-defined Aß42 oligomers, rich in ß-sheet structure, that form pores in lipid bilayers. According to mass spectrometry (MS) and size exclusion chromatography (SEC), the oligomeric state of Aß42 in this preparation is compatible with a hexamer. We have named this preparation SMemO, Stabilized Aß42 Membrane Oligomer. Noticeably, under the same conditions, Aß40 adopts a monomeric α-helical structure in equilibrium with heterogeneous and non-stable oligomers, which do not form pores. The fact that the stabilized Aß oligomers are formed specifically by Aß42, the most neurotoxic variant, is the first indication of their biological relevance.

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"IntSide: a web server for the chemical and biological examination of drug side effects"

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Abstract

Drug side effects (SEs) are one of the main health threats worldwide, and an important obstacle in drug

development. Understanding how adverse reactions occur requires knowledge on drug mechanisms at the molecular level. Despite recent advances, the need for tools and methods that facilitate side effect identification still remains.

Very recently, we presented a top-down approach to identify chemical and biological drug features that may be involved in the development of adverse drug reactions (Duran- Frigola & Aloy, 2013). We delimited the chemical and biological space for each compound by gathering molecular properties from major biomedical resources and carried out an enrichment analysis, associating more than 1,000 SEs with molecular features. On the biological side, we considered drug targets and off-targets, pathways, molecular functions and biological processes. From a chemical viewpoint, we included molecular fingerprints, scaffolds and chemical entities.

Here, we introduce a web server, named IntSide, which automates this analysis and enables the quick and easy access to our findings. Moreover, we further extend the method by integrating additional biological information, like protein interactions and disease-related genes, to facilitate mechanistic interpretations. IntSide is available at http://intside.irbbarcelona.org/.

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Group 10

"Cyclic peptides to modulate protein-protein interactions"

Sonia Ciudad,[a] Núria Bayó, [a] Monica Varese,[a] Jesús García,[a] Meritxell Teixidó,[a] Ernest Giralt.[a,b]

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Abstract

Vascular endothelial growth factors (VEGFs) belong to the platelet-derived growth factor supergene family, and they play central roles in the regulation of angiogenesis and lymphangiogenesis. VEGF-A, the major

factor for angiogenesis, binds to two tyrosine kinase receptors, VEGFR-1 (Flt-1) and VEGFR-2 (KDR/Flk-1), and regulates endothelial cell proliferation, migration, vascular permeability, secretion and other endothelial functions.1

VEGF-VEGFR is crucial not only for physiological angiogenesis from early embryonic to adult stages but also for pathological angiogenesis, such as in cancer.

Several anti-VEGF/VEGFR strategies have been developed, including soluble receptors, neutralizing antibodies to VEGF or VEGFRs, other inhibitors of VEGF/VEGFR interaction, such as small molecules inhibiting signal transduction of KDR and antisense oligonucleotides with therapeutic value for a variety of malignancies as well as for other disorders, used alone or in combination with other agents.2

In the present work, we present the EXORIS library, synthesized in Prof. Ernest Giralt's lab in order to be tested as possible candidates to modulate different kinds of PPIs. The conformational characterization of these cyclic hexapeptides is described.

The NMR conformational study reveals that the studied peptides have a C2 symmetric conformation. All the studied peptides have the Xaa-D-Pro bonds in the trans conformation. Moreover we could conclude that all of them adopt a conformation that contains two β-turns. This was determined by the presence of two hydrogen bonds between the residues preceding the D-Pro residues.

We have also tested these peptides in front of VEGF, in an NMR-based study, and we have found a hit that binds VEGF with mM affinity, which allows us to start a hit to lead optimization process in order to obtain better compounds that bind VEGF with higher affinity.

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"A Network approach to Spinal Cord Injury"

Laura Isús1, Jofre Tenorio-Laranga1, Mireia Herrando-Grabulosa2, Caty Casas2 and Patrick Aloy1,3

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Abstract

Spinal Cord Injury (SCI) represents a severe health problem associated with lifetime disabilities. Immediate cell death occurring after SCI is followed by a progressive death of neurons and degeneration. Depending on the severity and the proximity to the soma of the axonal injury, spinal motoneurons (MNs) may evolve to a retrograde degeneration reaction or to a regenerative process [1]. Combining proteomic data with physical and functional interaction information can provide further insights into the dynamic behavior and mechanisms involved in the degeneration and regeneration of spinal motoneurons. We propose a directed integrative approach to decipher the distinct molecular and cellular changes that contribute to each type of process and particularly in the death mechanisms and the

characteristic neuropatic pain associated with the degenerative process. Combining proteomic data with protein-protein interaction networks specifically containing disease-associated genes and their direct interactors, can help us to razionalize our findings and may provide new candidate and interesting proteins for further analyses.
Comparing GSEA analysis of our lists of candidates and networks with classical functional enrichment analysis (DAVID) we have been able to identify distinct enriched pathways (motives) between the degenerative and the regenerative process instead of general GO terms and KEGG pathways common to both models and many other disorders. In conclusion, some motives become siginificant only when direct interactors were included in the GSEA (e.g., Anoikis and Autophagosome fusion events) showing that by mapping our candidates to an interaction network we are increasing the statictical power of our analysis.

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"SEARCH, DEFINE AND STUDY OF HUMAN GENE REGIONS HIGHLY ENRICHED WITH CODONS RELATED WITH HETADAT"

ÀLBERT RAFELS, LLUÍS RIBAS DE POUPLANA

Abstract

The roll of RNA modification enzymes for translation efficiency is one of the most important aspects in the characterization of these type of enzymes and the concept of translation efficiency can be seen from many points of view. Here we focus on the tRNA modifying enzime ADAT2-ADAT3 present in most eukaryotes organisms including the hu- man. ADAT2-ADAT3 is an essential enzyme that allows Adenine (A) to Inosine (I) editing in the first anticodon position (position 34) by hydrolytic deamination [Keller, 1999]. We show that in the human transcriptome there are certain families of genes provided with ADAT stretches; which are regions inside the transcript enriched with codons whose cog- nates tRNAs might be previously modified by ADAT2-ADAT3. Moreover, a more detailed analysis of these ADAT stretches allows to estimate its size as well as to discern between the behaviour of each codon [Novoa et al., 2012].
Therefore we hypothesize how ADAT2-ADAT3 regulates the translation of these genes through the ADAT stretch regions.

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Group 11

"A chemo-centric view of human health and disease"

Miquel Duran-Frigola, David Rossell and Patrick Aloy

Abstract

Efforts to compile the phenotypic effects of drugs and environmental chemicals offer the opportunity to adopt a chemo-centric view of human health that does not require detailed mechanistic information. Here, we consider thousands of chemicals and analyze the relationship of their structures with adverse and therapeutic responses. Our study includes molecules related to the etiology of 934 health threatening conditions and used to treat 835 diseases. We first identify chemical moieties that could be independently associated with each phenotypic effect. Using these fragments, we build accurate predictors for approximately 400 clinical phenotypes, finding many privileged and liable structures. Finally, we connect two diseases if they relate to similar chemical structures. The resulting networks of human conditions are able to predict disease comorbidities, as well as identifying potential drug side effects and opportunities for drug repositioning, and show a remarkable coincidence with clinical observations.

"Synthesis of natural protein Sonic Hedgegoh"

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Abstract

Chemical synthesis of proteins is a powerful tool to synthetize small or medium proteins (30 kDa) with post-transcriptional modifications, such as glico-, phospho- and lipoproteins. Among all the reactions reported for the synthesis of proteins, Native Chemical Liagtion (NCL) (1) is the most employed nowadays to obtain large proteins chemically. The NCL is based on the chemoselective condensation of unprotected peptide fragments in aqueous solution at neutral pH. NCL present some requirements: one peptidic fragment may contain a thioester function at the C-terminal, while the other has to own a Cysteine residue at N-terminal. The key of this reaction is the synthesis of thioester peptides. In this project an alternative methodology will be used to obtain thioester; it is based on using N-acylureas like precursors (2,3) of peptide thioesters
This synthesis approximation will be used in the synthesis of a natural protein: Sonic Hedgehog (SHh). SHh is the most important protein in the Sonic Hedgegoh signaling pathway. It is implicated in important neuronal functions; moreover it is related to some cancer processes, what makes it a potential therapeutic target (4,5,6). Shh protein consists in 174 residues, it has a cholesterol molecule at C-terminal and a palmitic residue at N-terminal.

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"Rational design, synthesis and biophysical evaluation of peptide ligands for epidermal growth factor (EGF)"

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Human epidermal growth factor (EGF) is a small globular protein of 6.2 kDa that binds to the extracellular domain of EGF receptor (EGFR) and stabilizes its active dimeric conformation. This triggers the activation of the intracellular kinase domains and the initiation of the signal transduction cascade, resulting in cell growth and proliferation.

In numerous human carcinomas there is an overactivation of the EGF pathway. Thus, EGF-EGFR interaction is a well-known target for anti-cancer therapies. Several drugs that block the receptor activation (i.e. cetuximab and erlotinib) are currently in the market and a number of new agents are being developed to overcome current limitations such as drug-resistance and side effects. These drugs all target the receptor. However we believe that research on EGF blockers may identify a new class of drugs for cancer treatment.

Relying on existing structural data (X-ray and NMR structures) and using existent computational tools, such as docking and molecular dynamics, our goal is to design and synthesize a range of peptides that can potentially target EGF and prevent its interaction with EGF. In addition, we plan to use our group's expertise in biophyisical techniques to carry out an *in vitro* evaluation of their binding properties.

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Group 12

"Structural characterization of T7 bacteriophage DNA packaging machinery"

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Abstract

Herpes viruses are a leading cause of human viral diseases like Kaposi's sarcoma, mononucleosis or chickenpox. Serious neurological complications have been reported for both primary and reactivated infections [1]. During herpes virus infection cycle the viral genome is replicated in the cell nucleus in a concatameric form. After that, the DNA packaging machinery carries out the role of encapsidating unit-length genomes into preassembled capsids [2]. Given that the viral double-stranded DNA (dsDNA) packaging mechanism is well conserved in certain dsDNA bacteriophages and in herpes viruses, bacteriophage DNA packaging proteins can be used as models to figure out how the herpes virus packaging machinery works. These molecular machines have two components: the terminase and the connector or portal. The terminase complex is an ATP-driven molecular motor that cleaves the concatameric viral DNA and allows its incorporation into preformed capsids. The connector, located at a unique capsid vertex, is proposed to act as the entry point for the genome during encapsidation and the docking site for the terminase [3] [4]. Moreover, the connector is also important during assembly of the tail proteins and injection of the viral genome into the host cell [5]. Atomic models of dodecameric ring-shaped connectors of Φ29, SPP1 and P22 bacteriophages were obtained by X-ray crystallography [6] [7] [8]. Despite their size difference a conserved central domain was identified [9]. A model for the three-dimensional structure of T7 connector (codified in the gp8 gene) was obtained at 8 Å resolution, using single particle cryo-electron microscopy [10]. However, no crystallographic model of this protein is available so far. We have expressed the gp8-His protein in Escherichia coli, and after that we have purified it using a two-step chromatography procedure: immobilized metal ion affinity chromatography and size exclusion chromatography. Two peaks eluted from the size exclusion column, with a concentration-depending equilibrium established between both of them. Four conditions giving crystals have been found and optimized, and a dataset has been collected with diffractions spots up to 5.09 Å resolution. The seleno-methionine derivative protein has also been produced and purified, and in this case only one peak elutes from the size exclusion column. Seleno-methionine derivative crystals coming from different crystallization conditions have been diffracted with a maximum resolution of 10 Å. Analysis of the data obtained so far, further optimization of the crystals and finding of new crystallization conditions are required to obtain the atomic model of the protein.

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"Bike peptides: a ride through the membrane"

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Abstract

There are several examples in nature of cyclic peptides which display a biaryl or biaryl ether motif in their structures. Among them, complex polycyclic compounds with interesting biological activities such as vancomycin or aciculitins are found [1,2]. This common feature acts as a conformational constrain rendering the desired rigidity, as well as, enhancing their metabolic stability, potency and selectivity [3]. Furthermore, the biaryl motif provides a site for aromatic-aromatic or π-cation interactions with the residues present on protein surfaces.
Our goal is to synthesize medium-sized biaryl bicyclic peptides containing 5 or 6 amino acids which display a biaryl bond between the side-chains of two aromatic residues in the sequence. This novel kind of biaryl stapled-like peptides can be used as scaffolds and be decorated with the most suitable residues so as to allow recognition between them and a protein target.
Regarding the permeability of these compounds, the introduction of positively charged residues and the presence of the hydrophobic biaryl bridge are expected to favour their cellular uptake.
Guanidinium groups present in the side-chain of arginine-containing peptides also became interesting for us in order to target p53 tetramerization domain. According to previous experience in our group, calixarenes with this feature were able to act as "pharmacological chaperones" by interacting with p53 and different mutants stabilizing the native tetrameric assembly of the proteins [4]. This fact is highly relevant, since mutations in this domain leads to the development of different types of cancer due to the loss of the structure and/or activity of the proteins.

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"Urea & protein unfolding: from NMR to MD simulation"

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Abstract

The delicate equilibrium between the folded and functional structure of a protein and its unfolded state is highly dependent on environmental variables such as the solvent. For example the co-solvent urea is a protein denaturant that displaces the equilibrium towards unstructured and non-functional conformations of proteins. However the molecular mechanism behind its ability remains an enigma leading to ambiguous experimental interpretation. In this work we characterize structural,

dynamics, and energetics of the end-point of the unfolding process: we study an ensemble of conformations of ubiquitin, a small prototypical protein, unfolded in urea, using multi-replica molecular dynamics simulations in combination with NMR and SAXS data.

The analyses of the interactions between the unfolded proteins with both urea and water, lead us to conclude that the energetics stabilizing unfolded structures rely on dispersion interactions, mainly between protein apolar side chains and urea molecules.

Group 13

"Lyn kinase as regulator of LPS-triggered TLR4 pro-inflammatory signaling"

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Abstract

Toll-like receptor 4 (TLR4) of macrophages is activated by lipopolysaccharide (LPS), a constituent of Gram-negative bacteria. During bacterial infection LPS triggers pro-inflammatory reactions which can lead to sepsis. Activation of TLR4 involves a sequential engagement of serum LPS-binding protein, plasma membrane CD14 and the TLR4/MD2 signaling complex. CD14 is a GPI-anchored protein of plasma membrane rafts which can also accommodate Lyn, a member of the Src tyrosine kinase family. As the role of protein tyrosine phosphorylation in TLR4 signaling has been indicated, we undertook studies to establish whether Lyn kinase modulates LPS-induced pro-inflammatory responses of macropahges.

We found that stimulation of RAW264 cells with 1-1000 ng/ml LPS induced activation of Lyn indicated by phosphorylation of Tyr396 and dephosphorylation of Tyr507. Activated Lyn was enriched in the raft fraction of the plasma membrane due to palmitoylation and the integrity of rafts was crucial for the enzyme activity. To reveal the role of Lyn in TLR4 signaling, a series of Lyn constructs fused with GFP at the C-terminus was obtained: wild type Lyn, constitutively active Y507F so-called Lynup/up, kinase-dead K275R Lyn with point mutation in the catalytic domain SH1 and Lyn with point mutations in either SH2 (R155A) or SH3 (W98A) domains. All Lyn constructs were overexpressed in RAW264 cells with 40% efficiency and became anchored in the plasma membrane, as shown by confocal microscopy. Cellular level of LynWT and Lynup/up was up-regulated in LPS-stimulated cells due to inhibition of their proteasomal degradation. Concomitantly, production of TNF-α and RANTES, two cytokines chosen as readout for MyD88 and TRIF-dependent signaling of TLR4, was markedly inhibited. Mutation of SH2 or SH3 domains abolished this inhibitory effect of Lyn, while kinase-dead Lyn K275R up-regulated significantly production of both cytokines at mRNA and protein level. Looking for mechanisms of the regulatory function of Lyn we found that the enzyme affected activity of transcription factor NFκB and MAP kinases, the main effectors of TLR4 controlling expression of pro-inflammatory genes. The enzymatically active kinase forms down-regulated activity of NFκB. On the other hand, due to an ability to interact with PI3-kinase via SH2 and

SH3 domains, Lyn suppressed phosphorylation of MAP kinases p38 and ERK, but not JNK.
These data indicate that both the catalytic activity of Lyn and its ability to interact with other proteins via SH2 and SH3 domains contribute to the final outcome of the Lyn involvement in TLR4 signaling.Literature

"Dissecting the functions of g-TuRC subunits in microtubule nucleation and organization"

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Abstract

The g-TuRC is made up of 13 molecules of g-tubulin associated with multiple g-tubulin complex proteins (GCPs2-6) the targeting factor GCP-WD/NEDD1, and the two new members GCP8 and MOZART1. GCP8 has a role in interphase microtubule organization while MOZART1 was shown to have a role in spindle microtubule organization. Depletion of g-tubulin or any of the GCPs desestabilize g-TuRC in sucrose gradient, but only g-tubulin, GCP2, and GCP3, which form a smaller subcomplex (gTuSC), are essential in yeast and flies. While the role of g-TuRC in the nucleation of microtubules is well known, the molecular mechanisms of g-TuRC assembly are unknown. In this work we analyze and compare systematically the role of g-TuRC subunints in g-TuRC assembly and function. We analyze the individual role of g-TuRC subunits in the ability to recruit g-tubulin to the centrosome, centriole duplication and mitotic cell progression by immunofluorescence microscopy of HeLa cells treated with siRNA of each subunit. The stability of the gTuRC subunits where determined by western blotting. To analyze the state of gTuSC and gTuRC we performed sucrose gradient fractionation with the cells treated with siRNA. The sucrose gradient showed a shift to smaller peaks of both g-TuSC subcomplex and g-TuRC in GCP3 depletion compared to the control. The depleted cells exhibit increased mitotic index (40.04% in GCP2 depletion, 33.27% in GCP3 depletion vs 3.04% in control), most GCP3 cells have unseparated centrosomes. The depletion of GCP4, GCP5 and GCP6 did not destabilize the gTuSC components, but gTuRC stability on gradients was strongly affected, in particular by GCP6 depletion. This correlated with an increased mitotic index in GCP6 depleted cells (35%). All together, these results suggest that in human cell line the GCP6-dependent assembly into g-TuRC is essential to support the essential g-tubulin-dependent functions.

"y^+LAT-1: unravelling its role in immune complications"

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Abstract

SLC7A7 encodes for the amino acid transporter y+LAT-1, which is mainly expressed in the epithelial cells of kidney and intestine, mediating the transport of cationic amino acids (aa+). Its lack results in an autosomic recessive disease known as Lysinuric Protein Intolerance (LPI, MIM 222700),

characterized by a defect in the urea cycle, malabsorption and altered reabsorption of aa+1. Some LPI patients in addition develop immunological complications, and interestingly y+LAT-1 is also expressed in some cells of the immune system, although its contribution is still unknown2.
Due to ablation of SLC7A7 is embryonically lethal in mouse3, our group recently has generated the first conditional knock out mouse model for y+LAT-1 (SLC7A7-/-). It reproduces perfectly the human LPI symptoms. Now, we are checking if our mouse model also develops the associated immune disorders.
One of these immunological alterations is Pulmonary Alveolar Proteinosis (PAP, MIM 610910), a life threatening disease caused by a fault in the catabolism of lung surfactant by alveolar macrophages (AM)4. There are two features that clearly reveal PAP: accumulations of surfactant inside the alveoli, and a population of enlarged AM which shows a foamy phenotype4,5.
Histology of SLC7A7-/- lungs revealed PAS positive staining and PAS-diastase resistant material, indicating the accumulation of lung surfactant in the alveoli. To assess the AM population, bronchoalveolar lavages (BAL) of the lungs were performed. It has been reported that around 80-90% of the cells obtained from BAL are AM6. SLC7A7-/- AM population shows higher number of "foamy" cells and bigger area compared with the control ones.
In conclusion, SLC7A7-/- mouse model also reproduces some immune like disorders of human LPI, so it will allow us to study the molecular mechanisms of this alterations.

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Group 14

"Immortalized Human Renal Epithelial Cells For Bioartificial Kidney"

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Abstract

It is now recognized that renal diseases are global public health problems, with the incidences of end-stage renal diseases (ESRD) rising annually. ESRD requires long-term kidney replacement therapy. Due to the lack of donor kidneys, most of ESRD patients depend on dialysis treatment using either artificial kidney (haemodialysis, HD) or the peritoneal membrane (peritoneal dialysis, PD). Both modes are inefficient in removing uremic waste molecules and excess body fluids contributing significantly to severe patient health problems, poor life quality and high mortality (15-20%).
The main goal of the project is the development of a prototype bio-artificial kidney device that utilizes human renal epithelial cells for removal of uremic toxins. Precisely, it will focus on the full characterization of renal epithelial cells which are conditionally immortalized proximal tubule cells (ciPTEC) obtained from healthy donors, for which four different lines are available.
The quality of these cells will first be evaluated by the assessment of their biomarker expression profiles when grown on natural extracellular matrix coatings under uremic and non-uremic conditions. Next, ciPTEC monolayers on the bioactive membranes will be subjected to histomorphological analysis and biomarker expression analysis. The formation of tight monolayers will be assessed by electron microscopy analysis of cell-cell junctions (tight junctions, occluding junctions) and cell-extracellular matrix contacts. Up to now, monolayers formation has been tested using two different Polyethersulfone(PES)-based hollow fiber membranes (MICROPES and HCO1100). The optimal coating combination has been established: a first layer of L-3,4-dihydroxyphenylanine (L-DOPA) and a second layer of human collagen IV. MICROPES revealed to be better than HCO1100, although seeding procedure needs to be optimized.
The next step will be the assessment of ciPTECs functionality with respect to organic anionic uremic toxins transport.

"CNS Friedreich's Ataxia Gene Therapy BBB-shuttles bioconjugation to HSV-1 particles"

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Abstract

The blood-brain barrier (BBB) is the principal pathway of molecular exchange between the central nervous system (CNS) and the rest of the body. As the brain could be considered the most important organ and also fragile, there is an exhaustive control in the in- and outtake of the surrounding compounds. This task is carried out by the BBB.
Many diseases affect the CNS. Despite of that, in many cases the cure is not reached or the treatments are not satisfactory. One common trouble for all of them is that once an effective drug for its treatment is achieved, it cannot cross through the BBB. Friedreich's Ataxia (FA) could be included into those diseases, being the most prevalent inherited ataxia in Europe and US, caused by an expression of a nonfunctional mitochondrial protein, Frataxin (FXN), in all the cells, but affecting mainly CNS and heart.

In order to solve that problem, there is a general approach in which is working the group of Dr. E. Giralt, the BBB-shuttles. These molecules are peptides able to cross the BBB through different mechanisms and carry different cargos that cannot cross unaided. There are different mechanisms by which a molecule could reach the CNS. The BBB-shuttles designed to cross through receptor-mediated transcytosis allow the transport of huge cargos, like proteins, antibodies or gold nanoparticles1.

We work in a gene therapy treatment for FA by using modified Herpes Virus Simplex type 1 (HSV-1) particles, containing an infectious artificial bacterial chromosome (iBAC). These viral particles allow a long and stable expression of the whole gene of FXN (135 kb).2 Hence, the different isoforms of FXN protein are expressed in the infected cells. Despite its advantages, HSV-1 particles are not able to cross the BBB and therefore they cannot access the CNS, the principal affected organ by FA, together with the heart. Thus, BBB-shuttles are coupled to these particles, through bioconjugation methodologies, giving HSV-1 access to the CNS.

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Poster walks groups

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	Jessica Vitos
	Lada Murcia
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	Hilda-Yuliana Enciso-Salas
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	Susana Barros
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	Craig Donoghue
	Dan Byrom
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	Jurgen Walther
	Diana Buitrago
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Group 11

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Group 14

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IRB BARCELONA

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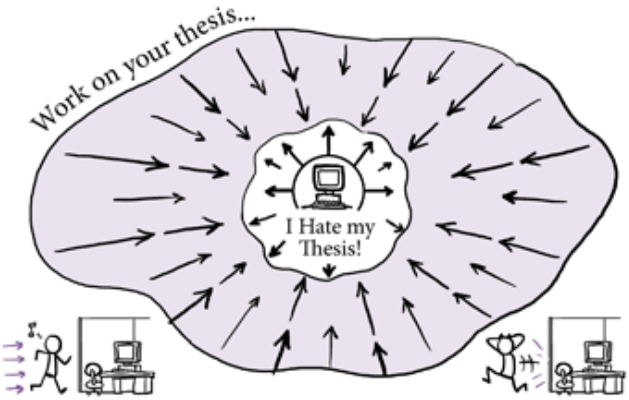
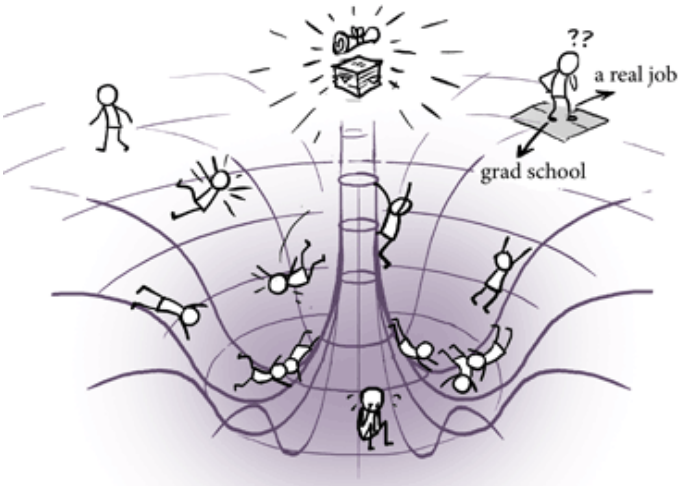
THE THESIS REPULSOR FIELD

The Thesis Repulsor Field (TRF) is a generalized model of the forces experienced by an individual in the final stages of graduate space-time*.

It is characterized by an attractor vector field directed towards completion of the thesis but with an intense repulsive singularity at its origin.



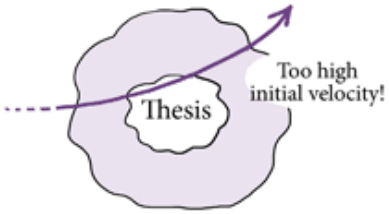
The resulting potential well of wasted potential acts as grad students follow the gradient in the perceived direction of least work:



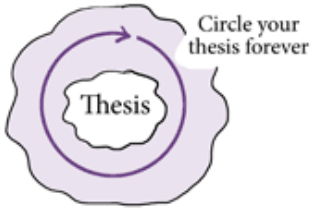
In reality, TRF is not an actual force, but rather a distortion of the mindspace continuum, in which grad students are simply responding to the curvature of their own neuroses.

Several trajectories are possible due to this vector field:

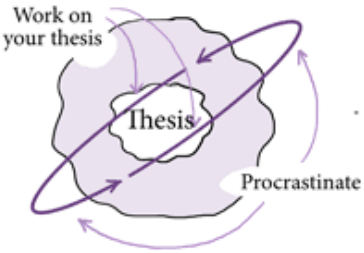
Complete Repulsion



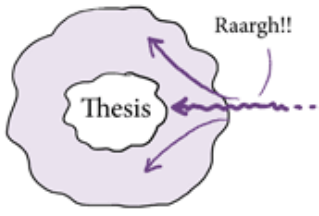
Infinite Orbit



Periodic Productivity



Grin and bear it



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*Graduate space-time is just like real space-time, but with added imaginary dimensions.

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