cnio - CaixaResearch
FRONTIERS
MEETINGS

Madrid 22nd – 23rd May 2023

Genome organisation and stability

Organising committee

Felipe Cortés
Spanish National Cancer Research Centre – CNIO, Spain

Óscar Fernández-Capetillo
Spanish National Cancer Research Centre – CNIO, Spain

Ana Losada
Spanish National Cancer Research Centre – CNIO, Spain

Andre Nussenzweig
National Institutes of Health – NIH, US

Speakers

Brittany Adamson
Princeton University, US

Fred Alt
Harvard Medical School, US

Luis Aragón
London Institute of Medical Sciences, UK

Camilla Björkegren
Karolinska Institute, Sweden

Maria A. Blasco
Spanish National Cancer Research Centre - CNIO, Spain

Keith Caldecott
University of Sussex, UK

Victor Corces
Emory University School of Medicine, US

Gaelle Legube
Centre de Biologie Intégrative, France

Peter McKinnon
St Jude's Children Research Hospital, US

Jan-Michael Peters
Institute of Molecular Pathology, Austria

Ana Pombo
Max Delbrück Center for Molecular Medicine, Germany

Marie-Noelle Prioleau
Institut Jacques Monod, France

Vassilis Roukos
Institute of Molecular Biology Mainz, Germany

Kikuë Tachibana
Max Planck Institute of Biochemistry, Germany

EXCELENCIA SEVERO OCHOA 2012 - 2023
GOBIERNO DE ESPAÑA
MINISTERIO DE CIENCIA E INNOVACIÓN
Instituto de Salud Carlos III
cnio stop cancer
ARIMA GENOMICS
"la Caixa" Foundation

Madrid 22nd – 23rd May 2023

Genome organisation and stability

Spanish National Cancer Research Centre (CNIO)
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MEETINGS

Madrid 22nd – 23rd May 2023

Genome organisation and stability

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Madrid 22nd – 23rd May 2023

Genome organisation and stability

Summary

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Madrid 22nd – 23rd May 2023

Genome organisation and stability

Spanish National Cancer Research Centre (CNIO)
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MEETINGS

Madrid 22nd – 23rd May 2023

Genome organisation and stability

Organisers and Speakers

cnïo *stop cancer*

Madrid 22nd – 23rd May 2023

Genome organisation and stability

Venue:

Spanish National Cancer Research Centre – CNIO Auditorium, Madrid.

Organisers & Speakers

Felipe Cortés
Spanish National Cancer Research Centre – CNIO, Spain

Oscar Fernández-Capetillo
Spanish National Cancer Research Centre – CNIO, Spain

Ana Losada
Spanish National Cancer Research Centre – CNIO, Spain

Andre Nussenzweig
National Institutes of Health – NIH, US

CNIO - CaixaResearch Frontiers Meeting

Speakers

Brittany Adamson
Princeton University, US

Fred Alt
Harvard Medical School, US

Luis Aragón
London Institute of Medical Sciences, UK

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FRONTIERS
MEETINGS

Madrid 22nd – 23rd May 2023

Genome organisation and stability

Programme

cnio stop cancer

Monday May 22nd 2023

08.45-09.15 *Registration (main hall)*

09.15-09.45 Welcome address and opening talk
Neuronal genome stability and plasticity
Andre Nussenzweig,
National Cancer Institute, NHI/NCI, Bethesda, US

09.45-12.30 THE 3D GENOME I
Chair: Ana Losada

09.45-10.15 *Mechanisms of transgenerational epigenetic inheritance*
Víctor Corces,
Emory University School of Medicine, Atlanta, US

10.15-10.45 *Long-term changes in 3D genome structure at the onset of drug addiction*
Ana Pombo,
Max Delbrück Center for Molecular Medicine, Berlin, Germany

10.45-11.15 *Coffee break & poster session (social room)*

11.15-11.45 *How cohesin folds the genome by loop extrusion*
Jan Michael Peters,
Institute of Molecular Pathology, Vienna, Austria

11.45-12.00 *Cohesin inheritance across mitosis: mechanisms and functions*
[short talk] - ***Carlos Perea-Resa,***
Centre for Molecular Biology, CBMSO, Madrid, Spain

12.00-12.30 *Single molecule analysis of eukaryotic SMC complexes; Cohesin, Condensin and Smc5/6*
Luis Aragón,
London Institute of Medical Sciences, UK

12.30-14.00 *Lunch break (cafeteria)*

Monday May 22nd 2023

14.00-15.30 THE GENOME IN HEALTH AND DISEASE I
Chair: Camilla Björkegren

14.00-14.30 *IgH and Igk loci employ different mechanisms for loop-extrusion mediated RAG chromatin scanning*
Frederick Alt,
Harvard Medical School, Boston, US

14.30-14.45 *Whole genome doubling drives oncogenic loss of chromatin segregation*
[short talk] ***Ruxandra Lambuta,***
Swiss Institute for Experimental Cancer Research ISREC, Lausanne, Switzerland

14.45-15.15 *Telomere-originated genomic instability at the origin of cancer and aging*
Maria Blasco,
Spanish National Cancer Research Centre, CNIO, Madrid, Spain

15.15-15.30 *Structural basis for the inactivation of cytosolic DNA sensing by NHEJ proteins by the vaccinia virus*
[short talk] ***Oscar Llorca,***
Spanish National Cancer Research Centre, CNIO, Madrid, Spain

15.30-16.15 *Group picture (main entrance) Coffee break & poster session (social room)*

Monday May 22nd 2023

16.15-17.30 GENOME DYNAMICS AND STABILITY I
Chair: Juan Méndez

16.15-16.30 *Rewiring of the molecular mechanisms of DNA replication between naive and primed mouse embryonic stem cells*
[short talk] ***Patricia Ubieto,***
Spanish National Cancer Research Centre, CNIO, Madrid, Spain

16.30-17.00 *Dimeric G-quadruplex motifs determine a large fraction of strong replication origins in vertebrates*
Marie-Noëlle Prioleau,
Jacques Monod Institute, Paris, France

17.00-17.15 *Functional architecture of replication fork protection*
[short talk] ***Rodrigo Bermejo,***
Center for Biological Research, CIB-CSIC, Madrid, Spain

17.15 -17.30 *Replication-transcription crosstalk and genome stability*
[short talk] ***María Gómez,***
Centre for Molecular Biology, CBMSO, Madrid, Spain

17.30-18.45 *Poster session – Snack for all participants (social room)*

Tuesday May 23rd 2023

09.15-12.15 GENOME DYNAMICS AND STABILITY II

Chair: Oscar Fernández-Capetillo

09.15-09.30 *The role of cohesin in replication fork plasticity upon genotoxic treatments*
[short talk] ***Daniel González-Acosta,***
Institute of Molecular Cancer Research, Zürich, Switzerland.

09.30-10.00 *Chromosome and chromatin dynamics at DNA Double Strand Breaks*
Gaëlle Legube,
Centre de Biologie Intégrative, Toulouse, France

10.00-10.30 *The role of topoisomerase 2 in remodeling chromosome organization*
Vassilis Roukos,
Institute of Molecular Biology, Mainz, Germany

10.30-10.45 *DNA Topoisomerase 1-induced DNA Double Strand Breaks Associated to Gene Transcription*
[short talk] ***Fernando Gómez-Herreros,***
Institute of Biomedicine, IBIS, Sevilla, Spain

Tuesday May 23rd 2023

10.45-11.15 Coffee break & poster session (social room)

11.15-11.30	*Pol theta-dependent compromised DNA repair fidelity in embryonic stem cells* *[short talk]* ***Ophélie Martin,*** *University of Sussex, Brighton, UK*
11.30-12.00	*Mapping DNA damage repair mechanisms in human cells with high-resolution functional genomics* ***Brittany Adamson,*** *Princeton University, New Jersey, US*
12.00-12.15	*Towards a genetic atlas of human double-strand-break repair* *[short talk]* ***Felipe Cortés-Ledesma,*** *Spanish National Cancer Research Centre, CNIO, Madrid, Spain*

12.15-14.00 Lunch break (cafeteria)

Tuesday May 23rd 2023

14.00-15.30 THE 3D GENOME II

Chair: Gaëlle Legube

14.00-14.30 *Loop-extruding Smc5/6 organizes transcription-induced positive DNA supercoils*
Camila Björkegren,
Karolinska Institute, Solna, Sweden

14.30-14.45 *Genome control by cohesin*
[short talk] ***Roel Oldekamp,***
Netherlands Cancer Institute, Amsterdam, Netherlands

14.45-15.00 *Specific functions and regulation of cohesins STAG1 and STAG2*
[short talk] ***Ana Losada,***
Spanish National Cancer Research Centre, CNIO, Madrid, Spain

15.00-15.30 *Mechanisms of genome "awakening" and 3D genome reorganization at the start of life*
Kikuë Tachibana,
Max Planck Institute of Biochemistry, Martinsried, Germany

15.30-16.00 Coffee break & poster session (social room)

Tuesday May 23rd 2023

16.00-17.15 THE GENOME IN HEALTH AND DISEASE II

Chair: Felipe Cortés

16.00-16.30 *Chromatin architecture at susceptible gene loci characterizes DNA damage-induced neurodegeneration*
Peter McKinnon,
St Jude's Children Research Hospital, Memphis, US

16.30-17:00 *PARP1 protein in DNA Repair and Human disease*
Keith Caldecott,
University of Sussex, UK

17.00-17.15 *Deciphering the mechanism of toxicity by nucleolar stress*
[short talk] ***Oscar Fernández-Capetillo,***
Spanish National Cancer Research Centre, CNIO, Madrid, Spain

17.15-17.45 Awards for best posters and best short talks – farewell

Madrid 22nd – 23rd May 2023

Genome organisation and stability

Monday May 22th 2023

Session #1
THE 3D GENOME I

Chairperson: ***Ana Losada***

Neuronal genome integrity and plasticity

Andre Nussenzweig
Laboratory of Genome Integrity (LGI), National Cancer Institute (NCI)
National Institutes of Health (NIH)

The DNA of neurons is continually damaged due to lifelong, high-level metabolic and transcriptional activity. In addition, recent studies have demonstrated extensive "programmed" DNA damage in differentiating post-mitotic neurons. Here, we identify these endogenous lesions as single strand break intermediates of thymine DNA glycosylase (TDG)-mediated removal of oxidized methylcytosines at neuronal enhancers. Interrupting active DNA demethylation using anti-neoplastic cytosine analogs triggers TDG-dependent neuronal cell death associated with massive chromosomal rearrangements of neuronal enhancers. This suggests that the well-known neurotoxic side effects of certain chemotherapies, also called "chemobrain," could be linked to DNA repair processes intrinsic to normal neuronal differentiation. We will discuss recent studies that describe the interplay between programmed DNA damage, cell fate specification and mutagenesis in post-mitotic neurons.

Notes

Mechanisms of transgenerational epigenetic inheritance

Victor G. Corces
Professor. Department of Human Genetics
Emory University School of Medicine
Atlanta, US

The mechanisms by which epiphenotypes are transmitted transgenerationally in mammals are poorly understood. Exposure of pregnant mouse F0 females during E7.5-E13.5 to bisphenol A results in obesity in the F2 progeny in the absence of additional exposure. This epiphenotype can be transmitted through the male and female germlines up to the F5 generation, decreases in F6, and disappears in F7. Analyses of chromatin changes in the sperm of the F1 generation reveal a widespread increase in chromatin accessibility at binding sites for CTCF and other transcription factors accompanied by alterations in 3D organization. Comparison of the transmission of obesity between F2 and F5 and its disappearance in F7 with alterations in the binding of these transcription factors points to the recruitment of CTCF to a new site located in an intron of the Fto gene and activation of two different enhancers that interact with Irx3 and Irx5 in a BPA-dependent manner. Deletion of the CTCF site using CRISPR-Cas9 results in mice that fail to gain weight after ancestral BPA exposure. Observations from Hi-C data suggest that the two enhancers form an autoregulatory feedback loop that, in combination with a decrease of m6A in sperm enhancer RNAs, may cause alterations of Irx3 and Irx5 gene expression in the embryo after fertilization. Irx3 and Irx5 are involved in the differentiation of appetite controlling AgRP/NPY neurons in the arcuate nucleus of the hypothalamus. Single-nucleus and immunofluorescence analyses suggest that exposure to BPA results in expansion of the number of orexigenic AgRP neurons. This expansion correlates with increased accessibility of the Fto proximal enhancer in radial glia-like neural stem cells (RG-NSCs), which give rise to AgRP/NPY neurons, and in mature oligodendrocytes. The results suggest that both genetic and epigenetic alterations of the same gene can lead to the same phenotypic outcomes on human health.

Notes

Long-term changes in 3D genome structure at the onset of drug addiction

Ana Pombo
Berlin Institute for Medical Systems Biology
Max Delbrück Centre for Molecular Medicine
Humboldt University of Berlin, Berlin, Germany

The three-dimensional (3D) structure of chromosomes is associated with gene regulation and cell function. Understanding how 3D genome structure varies between cell types and states, in development and in disease, promises to enhance the interpretation of genome sequence variation and to accelerate the discovery of disease target genes. To explore 3D genome structure variation in highly specialised cells of the brain, we applied Genome Architecture Mapping in different cell types from the adult murine brain: oligodendroglia (OLGs) from the cortex, dopaminergic neurons (DNs) from the midbrain and pyramidal glutamatergic neurons (PGNs) from the hippocampus. We found extensive cell-type specialisation of 3D chromatin contacts, such as extensive reorganisation of topological domains (TADs) and eu/heterochromatic compartments. We also discovered large scale decondensation events, or 'melting', of long genes when most highly expressed, many with roles in neurodevelopmental and neurodegeneration disorders. Through integration of 3D genome structure with single-cell expression and single-cell chromatin accessibility, we found PGNs and DNs form specific hubs of contacts containing genes associated with their specialised functions, such as addiction and synaptic plasticity, respectively. Our recent work explores the effects of environmental insults, such as sleep deprivation or a single administration with cocaine, on the complex chromatin folding of brain cells, to explore pathways regulated by genome architecture.

Notes

CTCF is a DNA-tension-dependent barrier to cohesin-mediated DNA loop extrusion

Iain F. Davidson[1], Roman Barth[2], Maciej Zaczek[1], Jaco van der Torre[2], Wen Tang[1], Kota Nagasaka[1], Richard Janissen[2], Jacob Kerssemakers[2], Gordana Wutz[1], Cees Dekker[2], and **Jan-Michael Peters[1]**

Affiliations:
1 Research Institute of Molecular Pathology; Vienna BioCenter, 1030 Vienna, Austria
2 Department of Bionanoscience, Kavli Institute of Nanoscience Delft, Delft University of Technology; Delft, Netherlands
3 Present address: Children's Cancer Research Institute, St. Anna Kinderkrebsforschung; 1090 Vienna, Austria

In eukaryotes, genomic DNA is extruded into loops by cohesin. By restraining this process, the DNA-binding protein CTCF generates topologically associating domains (TADs) that play key roles in gene regulation and recombination during development and disease. How CTCF establishes TAD boundaries and to what extent these are permeable to cohesin is unknown. To address these questions, we visualize interactions of single CTCF and cohesin molecules on DNA *in vitro*. We show that CTCF is sufficient to block diffusing cohesin, possibly reflecting how cohesive cohesin accumulates at TAD boundaries, as well as to block loop-extruding cohesin, reflecting how CTCF establishes TAD boundaries. CTCF functions asymmetrically, as predicted, but unexpectedly is dependent on DNA tension. Moreover, CTCF regulates cohesin's loop extrusion activity by changing its direction and by inducing loop shrinkage. Our data indicate that CTCF is not, as previously assumed, simply a barrier to cohesin-mediated loop extrusion but is an active regulator of this process, where the permeability of TAD boundaries can be modulated by DNA tension. These results reveal mechanistic principles of how CTCF controls loop extrusion and genome architecture.

Notes

SHORT TALK

Cohesin Inheritance across Mitosis: mechanisms and functions

Carlos Perea Resa; Estrella Isabel Sayago; Juan Giner; Irene Alvarez.

Severo Ochoa Molecular Biology Center, CBMSO-CSIC, Madrid. Spain

Proper development of multicellular organisms requires a delicate interplay between cell proliferation and differentiation to generate the range on cell types and functions. This cell diversity relies on differential gene expression while most of cell fate determination events require the passage through mitosis. How mitosis favors the rewiring on gene expression is not fully understood. Strikingly, most transcription is silenced early in mitosis due to the general release from chromatin of RNA polymerases and transcription factors. Understanding the molecular mechanisms that restore transcription once mitosis ends is key to decipher how cell fate is conserved or modified. The ring-shape complex cohesin is linked to RNA polymerase II activity and dynamic. Two distinct cohesin subcomplexes, defined by the presence of either STAG1 of STAG2 subunits, influence genome organization and expression. Early in mitosis, most cohesin complexes dissociates from chromatin to resolve sister chromatids and to favor transcription silencing while late in telophase cohesin re-associates with DNA. The mechanisms behind cohesin transmission from mother to daughter cells and its functional implications are mostly unexplored. We investigate the specific contribution of inherited cohesin on transcription resuming after mitosis. Based on the SNAP technology, we developed a cellular system to specifically label and track inherited cohesin. Results evidence recycling of both subcomplexes with cohesinSTAG1 showing a rapid chromatin reassociation during the mitotic onset compared to cohesinSTAG2. IPMS data revealed the specific enrichment in factors involved on transcription initiation and elongation among STAG1 interactors during mitosis. Finally, rapid inducible depletion of STAG1 or RAD21, using degron approaches, impairs proper levels and localization of RNA Pol II regulators. In summary, our work reveals a central role for inherited cohesinSTAG1 on transcription restart after mitosis.

Notes

Single molecule analysis of eukaryotic SMC complexes; Cohesin, Condensin and Smc5/6

Luis Aragón
DNA Motors
MRC London Institute of Medical Sciences
London, UK

Eukaryotic SMC complexes: cohesin, condensin and Smc5/6 complex, are a family of chromosomal ATPases that participate in many aspects of higher-order chromosome organization and dynamics. We have purified all three human and budding yeast SMC complexes and have studied their activities using optical tweezers. Using a quadrupole-trap system to hold two distinct DNA molecules we find that cohesin can hold DNA heteroduplexes in *trans* in addition to promote loop extrusion on individual molecules. We have also characterised the activity of Smc5/6 complex on ssDNA and dsDNA-ssDNA junctions. These results and their implication for the role of Smc5/6 in recombinational repair will be discussed.

Notes

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Genome organisation and stability

Monday May 22th 2023

Session #2
THE GENOME IN HEALTH AND DISEASE I

Chairperson: ***Camilla Björkegren***

IgH and Igk loci employ different mechanisms for loop-extrusion mediated RAG chromatin scanning

Frederick Alt
Harvard Medical School, Boston, US

Notes

SHORT TALK

Whole-genome doubling drives oncogenic loss of chromatin segregation

Ruxandra A. Lambuta[1,2,*], Luca Nanni[2,3,4,*], Yuanlong Liu[2,3,4], Juan Diaz-Miyar[1,2], Arvind Iyer[2,3,4], Daniele Tavernari[2,3,4], Natalya Katanayeva[1,2], Giovanni Ciriello[2,3,4,#], Elisa Oricchio[1,2,#]

1 Swiss Institute for Experimental Cancer Research (ISREC), School of Life Sciences, EPFL
2 Swiss Cancer Center Leman, Lausanne, Switzerland
3 Department of Computational Biology, University of Lausanne (UNIL), 1015 Lausanne, Switzerland.
4 Swiss Institute of Bioinformatics (SIB) Switzerland
* These authors equally contributed to this work
# Corresponding Authors

Whole genome doubling (WGD) is a recurrent genetic abnormality supporting cancer growth through its ability to promote chromosomal instability and aneuploidy. However, the chromatin organisation of WGD cells and its impact on tumour development and evolution have yet to be understood. In interphase, the chromatin is organized in a multi-layer 3D architecture of compartments, chromatin domains, and loops, which are associated with chromatin activity and alteration of this organization leads to aberrant phenotypes. In this study, we investigate how the chromatin is organized in cells that underwent WGD. We showed that in cells with a defective p53-dependent tumour suppressor pathway, WGD leads to loss of chromatin segregation (LCS), a phenomenon defined by increased contacts between otherwise well segregated chromatin structures. LCS was prompted by the cells' inability to upscale production of the architectural protein CTCF and the histone mark H3K9me3, both involved in chromatin structure maintenance. Activation of a p53-dependent tetraploid checkpoint or temporarily halting cell cycle progression restored protein production and resolved LCS in post-WGD cells. Longitudinal analyses demonstrated that post-WGD cells are able to form soft-agar colonies *in vitro* and tumours *in vivo*. In both cases, the tumorigenic cells presented aneuploid karyotypes, as well as repositioning of chromatin regions to different compartments compared to non-WGD cells. These compartment repositioning events were independent of chromosomal alterations, but were displaying epigenetic and transcriptional reprogramming affecting oncogenic loci. Remarkably, across experiments, the compartment repositioning events could be traced back to concordant changes already present in cells immediately after WGD, suggesting that regions of chromatin can be primed for repositioning upon tetraploidisation. Overall, our study reveals that chromatin evolution is a hallmark of WGD-driven cancers.

Notes

Telomere-originated genomic instability at the origin of cancer and aging

Maria Blasco
Scientific Director, CNIO
Head, Telomeres and Telomerase Group (CNIO)
Spanish National Cancer Research Centre, CNIO, Madrid, Spain

Telomeres are nucleoprotein complexes which protect the ends of linear chromosomes and which play a pivotal role in cellular and organismal ageing. Over the past two decades short telomeres have been associated with a large disease spectrum including degenerative diseases and cancer. In addition, a number of diseases known as telomeropathies or telomere sydromes, including some cases of aplastic anemia and pulmonary fibrosis, are linked to mutations in telomere maintenance genes. Our laboratory has made significant contributions to dissect the role of telomerase and telomere length as one of the key molecular pathways underlying cancer and aging. We previously demonstrated that telomerase activation by means of transgenesis as well as vector-based gene therapy delays a variety of age-related pathologies and increases survival in wild-type mice. Here, I will discuss our more recent work validating the effectivity of telomerase gene therapy for the treatment of diseases related to the presence of short telomeres including models for myocardial infarction, aplastic anaemia, pulmonary fibrosis and renal fibrosis

Notes

SHORT TALK

Structural basis for the inactivation of cytosolic DNA sensing via NHEJ proteins by the vaccinia virus

Angel Rivera-Calzada[1,2,*], Raquel Arribas-Bosacoma[3,*], Alba Ruiz-Ramos[1], Paloma Escudero-Bravo[1], Jasminka Boskovic[1], Rafael Fernandez-Leiro[1], Antony W. Oliver[4], Laurence H. Pearl[4,5], & **Oscar Llorca[1]**
*These authors contributed equally: Angel Rivera-Calzada, Raquel Arribas-Bosacoma.

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4 Genome Damage and Stability Centre, School of Life Sciences, University of Sussex, Falmer, Brighton BN1 9RQ, UK.
5 Division of Structural Biology, Institute of Cancer Research, Chester Beatty Laboratories, 237 Fulham Road, London SW1E 6BT, UK.

The Ku70-Ku80 heterodimer has a central role in the non-homologous end-joining (NHEJ) pathway of DNA repair in the nucleus, in which it recognizes DNA ends generated after double strand breaks in the DNA (dsDNA). After binding to dsDNA, Ku70-Ku80 forms a complex with the catalytic subunit of the DNAdependent protein kinase (DNA-PKcs) to assemble the DNA-PK holoenzyme.
Interestingly Ku70-Ku80 also functions as a cytosolic DNA sensor, forming a complex with DNA-PKcs after recognition of dsDNA. Then DNA-PK acts in STING-dependent and -independent pathways to activate an innate immunity response. Interestingly, Ku70-Ku80 detects dsDNA from viruses that replicate in the cytoplasm and activates an inflammatory response [1, 2], but some viruses have found ways to counteract this defence mechanisms. Two proteins from Vaccinia virus, C4 and C16, are the best characterized examples of viral proteins that inactivate Ku70-Ku80 dependent DNA sensing and enhance virulence, but the structural basis for their inactivation mechanism has remained unknown [3]. We have determined the structure of Ku70-Ku80 bound to C16 using cryo-Electron Microscopy (cryoEM) [4]. Ku70-Ku80 consists on a preformed ring that binds dsDNA, and C16 sterically blocks this access route preventing binding to a dsDNA end and the assembly of the DNA-PK holoenzyme, which averts signalling into the downstream innate immunity system. C4 mimics these activities using a domain with 54% sequence identity to C16. Sensing invasive cytosolic DNA is a key component of the innate immunity system against viral infection. Our results reveal how C4 and C16, two proteins with not fully redundant functions, evolved similar strategies to subvert the capacity of Ku70-Ku80 to recognize viral DNA. We also find that this mechanism is conserved in other poxvirus such as the causative agents of variola and monkeypox.

References
1. Hristova, D. B. et al. J. Gen.Virol.101, 1133–1144 (2020).
2. Chaplin, A. K. et al. Mol. Cell 81, 3400–3409 e3 (2021).
3. Scutts, S. R. et al. Cell Rep. 25, 1953–1965 e4 (2018).
4. Rivera-Calzada, A. et al. Nat. Commun. 13, 7062 (2022).

Notes

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Genome organisation and stability

Monday May 22th 2023

Session #3
GENOME DYNAMICS AND STABILITY I

Chairperson: ***Juan Méndez***

SHORT TALK

Rewiring of the molecular mechanisms of DNA replication between naive and primed mouse embryonic stem cells

Patricia Ubieto-Capella[1], Pilar Ximénez-Embún[2], Javier Muñoz[2], Juan Méndez[1]

1 DNA Replication Group, Molecular Oncology Programme,
2 Proteomics Unit- ProteoRed-ISCIII, Biotechnology Programme, Spanish National Cancer Research Centre (CNIO), Madrid, Spain

The dynamics of DNA replication are influenced by chromatin architecture and transcriptional activity and thus, are subject to change along differentiation and reprogramming. Differentiated cells display faster forks and fewer active origins than primed mouse embryonic stem cells (mESCs), which resemble the characteristics of the post-implantation epiblast. However, the DNA replication dynamics during earlier states of pluripotency are poorly understood. Primed mESCs can be de-differentiated *in vitro* into a naive pre-implantation-like state by inhibition of MEK and GSK3 kinases with small inhibitors (2i). Primed-tonaive mESC reprogramming triggers global DNA hypomethylation and reorganization of the chromatin to a more open configuration concurrently with upregulation of core pluripotency factors and downregulation of lineagecommitment genes.

We have found that naive mESCs display higher replication fork asymmetry, lower fork progression rate and a compensatory increase in origin usage compared to primed mESCs. Using iPOND ("isolation of proteins on nascent DNA") coupled to mass spectrometry we have characterized the replisome composition of mESCs at these two pluripotency states and we have identified differently enriched proteins that could contribute to the DNA replication rewiring occurring in the primed-tonaive transition.

Among the candidates enriched in naive mESCs replisomes are several components of the DNA damage response pathway, including MRE11 and RAD50 from the MRN complex. Our results indicate that MRE11 is recruited to the forks during the primed-to-naive transition in response to transcription-replication conflicts and modulates fork progression in naive mESCs. Finally, we will present data showing that MRE11 nuclease activity is required for the efficiency of primed-to-naive reprogramming.

Notes

Dimeric G-quadruplex motifs-induced NFRs determine strong replication origins in vertebrates

Jérémy Poulet-Benedetti[1], Caroline Tonnerre-Doncarli[1], Juliette Mandelbrojt[1], Franck Picard[2] and **Marie-Noëlle Prioleau[1]**

1 Université Paris Cité, CNRS, Institut Jacques Monod, F-75013 Paris, France.
2 Laboratory of Biology and Modelling of the Cell, Université de Lyon, Ecole Normale Supérieure de Lyon, CNRS, UMR5239, Université Claude Bernard Lyon 1, Lyon, France.

Replication of vertebrate genomes is tightly regulated to ensure accurate duplication, but our understanding of the interplay between genetic and epigenetic factors in this regulation remains incomplete. We investigated the involvement of three elements enriched at gene promoters and replication origins: guanine-rich motifs potentially forming G-quadruplexes (pG4s), nucleosome-free regions (NFRs), and the histone variant H2A.Z, in the firing of origins of replication in vertebrates. We showed that two pG4s on the same DNA strand (dimeric pG4s) are sufficient to induce assembly of an efficient minimal replication origin without inducing transcription. Dimeric pG4s in replication origins trigger formation of an NFR next to precisely-positioned nucleosomes enriched in H2A.Z on this minimal origin and genome-wide. Thus, our data suggest a crucial role for dimeric pG4s in the organization and duplication of vertebrate genomes. It supports the hypothesis that a nucleosome close to an NFR is a shared signal for the formation of replication origins in eukaryotes. We observed that early replicating fragile sites (ERFSs) correlate with the presence of short, highly transcribed genes forming R-loops and containing strong replication origins containing dimeric pG4s. We are currently investigating how a strong replication origin is functioning when located within the body of an actively transcribed gene. Our preliminary results show that strong origins can fire efficiently within the body of actively transcribed genes containing R-loops, without affecting the level of transcription. Moreover, we observed that the presence of two strong initiation sites closely spaced, an organization found at many promoters, have the capacity to advance the replication timing. We will now determine how this organization affects chromosome stability. We aim at providing important mechanistic insights into the prioritization of two potentially conflicting DNA-dependent processes and at better understanding the causes of ERFSs instability.

Notes

SHORT TALK

Functional architecture of replication fork protection

Dolores Jurado-Santiago, Mohammed Al Mamun, Ana González-Herrero, Juan Carlos Cañas, **Rodrigo Bermejo**

Margarita Salas Center for Biological Research (CIB-CSIC), Madrid, Spain

During chromosome replication parental DNA is unwound by replicative helicases to generate fork structures in which single-stranded templates are provided to DNA polymerases. These generate nascent strands with intrinsically different mechanisms: leading strands are synthesised continuously in the same direction of fork progression, while lagging strands are synthesised discontinuously through periodic priming of short Okazaki fragments, which are later processed and sealed together. In eukaryotes, replication also requires the disassembly of nucleosomes, in a process in which parental histones are actively directed to assemble nucleosomes in both leading and lagging strands to ensure that epigenetic information is transmitted to daughter cells. Replication forks have to negotiate different obstacles hindering their progression, from supercoiling generated during helix unwinding itself to proteins tightly bound to DNA or inter-strand crosslinks.

In addition, the continuity of DNA synthesis can be challenged by damaged templates, secondary structures assumed by single-stranded DNA or RNA-DNA hybrids forming upon conflicts with gene transcription. Multiple factors, including DNA topoisomerases, key replisome components, DNA repair proteins and specialized helicases assist replication progression to promote fork stability and thus avoid the formation of lesions altering genetic information and underlying cancer onset.

Notes

SHORT TALK

Replication-transcription crosstalk and genome stability

José Miguel Fernández-Justel, Cristina Santa-Maria, Alicia Gallego and **María Gómez**

Functional Organization of the Genome Group Centro de Biología Molecular Severo Ochoa (CSIC/UAM) Nicolás Cabrera 1, 28049-Madrid, Spain

Copying and decoding the genomic information in a timely and accurate fashion is essential for life. Both tasks (DNA replication and transcription) are remarkably complex in scale and regulation, and the molecular machineries involved in them translocate on the same chromatin template, often in opposite directions and at different rates. This impose cells to employ efficient mechanisms to coordinate both processes which, very likely, in turn influence many key parameters of cellular function, including genome organization, chromatin structure or mutagenesis rates. Recent work from our laboratory showed that appropriate RNA turnover in chromatin is essential to avoid replicative stress and limit replication-transcription conflicts, and unveiled an unexpected role of histone H1 on RNA posttranscriptional modifications (Almeida et al., 2018; Fernández-Justel et al., 2022; Gallego et al., 2022). In particular, we found that non-coding RNA retention in chromatin associates with reduced levels of m6A in histone H1-depleted cells, resulting in increased stability of chromatin-bound RNAs and R-loops accumulation, which in turn generates problems with incoming DNA replication forks.

Interestingly, accumulation of low-m6A RNAs on chromatin also occurs upon challenging WT cells with different chemotherapeutic drugs that generate DNA damage, like doxorubicin (doxo) or 5-fluorouracil (5-FU), raising the hypothesis that reductions of m6A levels on chromatin-RNAs might constitute a general response to cellular stress to dynamically reorganize chromatin architecture.

References:

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- Fernández-Justel et al., 2022, Cell Rep 40: 111329. (10.1016/j.celrep.2022.111329)
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Notes

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Genome organisation and stability

Tuesday May 23rd 2023

Session #4
GENOME DYNAMICS AND STABILITY II

Chairperson: ***Oscar Fernández-Capetillo***

SHORT TALK

The role of cohesin in replication fork plasticity upon genotoxic treatments

Daniel González-Acosta[1], Melani Martins-Rodrigues[1], Ana Losada[2], Massimo Lopes[1].

1. Institute of Molecular Cancer Research, Zürich, Switzerland.
2. Chromosome Dynamics Group, Centro Nacional de Investigaciones Oncológicas (CNIO), Madrid, Spain.

Replication of the genome is a highly regulated process, ensuring correct transmission of genetic and epigenetic information to daughter cells. This process is targeted by many chemotherapeutic agents, preventing growth of highly proliferative cells. Replication interference leads to replication stress (RS) and triggers a rapid cellular response, involving replication fork plasticity and checkpoint mechanisms regulating cell cycle progression.

One important layer for the regulation of DNA replication is related to genome architecture. DNA replicons inside topologically associating domains (TADs) replicate simultaneously appearing as broad replication domains in Repli-seq approaches. Initial studies identified a role for the cohesin complex regulating origin firing, but not fork progression within replication domains. High resolution replication profiles recently confirmed that acute depletion of cohesin de-localizes replication origins within early-replicating TADs.

However, little is currently known about how stressed replication forks are regulated by factors governing genome architecture. Studies in yeast and human cells showed that cohesin removal or transfer behind replication forks is essential to maintain proper fork progression and stability in unperturbed and challenged Sphase. Moreover, cohesin was reported to rapidly accumulate at stressed replication forks and assist fork stability, but the underlying molecular mechanisms have remained elusive.

Here, using mainly proximity ligation (PLA) and stretched DNA fiber assays, and taking advantage of available and newly-generated auxin-inducible degrons of cohesin subunits and associated factors, we found that cohesin is a master regulator of replication fork plasticity and progression upon clinically-relevant mild replication stress treatments. Moreover, we will present intriguing preliminary data, indicating that different cohesin functions are required for the response to different sources of RS.

Notes

Chromosome and chromatin dynamics at DNA Double Strand Breaks

Gaëlle Legube
CBI LBCMCP CNRS UMR5088
University of Toulouse, Toulouse, France

DNA double-strand breaks (DSBs) are highly toxic lesions that are rapidly repaired by two main pathways, namely Homologous Recombination (HR) and Non Homologous End Joining (NHEJ). Using a cell line, called DIvA (for DSB Inducible via AsiSI), where multiples breaks are induced at annotated positions, combined with genome-wide, high throughput sequencing based techniques (ChIP-seq, HiC...) we investigate the contribution of chromatin and chromosome conformation in the response to DSB. I will present our recent work, on the contribution of chromatin and Topologically Associating Domains (TADs) during DSB repair. More specifically we recently demonstrated the role of cohesin-mediated loop extrusion in establishing gH2AX on an entire TAD in cis to DSBs. We now provide evidence that when established, gH2AX "tagged" TAD further self-segregate in the nucleus, forming a novel DSB-induced chromatin compartment (the "D" compartment) that contributes to the activation of the DNA damage response.

This project has received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (grant agreement No 647344 and grant agreement No 101019963)

Notes

The role of topoisomerase 2 in remodeling chromosome organization

Vassilis Roukos

Assistant Professor of Biology at the Department of Medicine of the University of Patras, Greece
Group Leader at the Institute of Molecular Biology (IMB), Mainz, Germany

How spatial chromosome organization influences genome integrity is still poorly understood. We and others recently shown that DNA double-strand breaks (DSBs) mediated by topoisomerase 2 (TOP2) activities are enriched at chromatin loop anchors with high transcriptional activity, often localised within genomic hotspots that recurrently translocate in cancer. Given the localisation and activity of TOP2s at chromatin loop and TAD boundaries it is a plausible scenario that TOP2s are required to resolve topological issues arising from loop extrusion by eliminating topological barriers and DNA entanglements. We will discuss here our recent study to assess the contribution of TOP2s in remodeling chromosome organization by performing HiC, Micro-C and high-throughput FISH microscopy experiments across the cell cycle upon acute degradation of TOP2 expression using degron systems in human cells.

Notes

SHORT TALK

DNA Topoisomerase 1-induced DNA Double Strand Breaks Associated to Gene Transcription

Diana Rubio-Contreras[1,2], & **Fernando Gómez-Herreros[1,2]**

1. Instituto de Biomedicina de Sevilla, IBiS/Hospital Universitario Virgen del Rocío/CSIC/Universidad de Sevilla, Seville 41013, Spain.
2. Departamento de Genética, Universidad de Sevilla, Seville 41080, Spain.

DNA double-strand breaks (DSB) are the most cytotoxic DNA lesions. Their accurate repair is critical to prevent cell death and maintain genomic integrity.
Some physiological DSBs have been associated to the activity of DNA topoisomerases, essential enzymes that transiently cut the DNA to release the torsional stress associated to DNA metabolism. Type 1 DNA topoisomerases (e.g., TOP1) cut one strand during their reaction cycle and remain covalently bound to 3' DNA end before resealing it. However, these breaks can become abortive (irreversible) and trigger cellular repair pathways. Intriguingly, although abortive TOP1 cycles mainly generate DNA single-strand breaks (SSB), these can turn into DSBs by a poorly understood mechanism. More importantly, how these breaks are repaired and their effect on genome stability and cell death is still unclear. These TOP1-dependent DSBs can be induced with selective TOP1 poison camptothecin (CPT) or its derivatives, commonly used as anticancer drugs. Additionally, physiological TOP1 DSBs might constitute a key cause of neuronal death in patients that carry mutations in SSB repair machinery. In this work we have characterized the specific pathway associated to TOP1 DSB repair. Importantly, we have characterised for the first time the effect of these breaks on genome instability.
Our results have important implications for the understanding of the molecular mechanism of DNA damage-associated neurodegenerative diseases and for the use of TOP1 poisons in cancer therapy.

Notes

SHORT TALK

Pol theta-dependent compromised DNA repair fidelity in embryonic stem cells

Ophélie Martin[1*], Lyuba Chechik[2], Karen Meaburn[1], Audrey Furst[2], Evi Soutoglou[1]

1 Genome Damage and Stability Centre, School of Life Sciences, University of Sussex, Brighton, United Kingdom
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Genomes are spatially organized in interphase nuclei, with positioning patterns correlating with gene activity, histone modifications, replication timing, and DNA repair. Work from our lab have revealed that the nuclear position of a DSB determines the balance between error-free and error-prone DNA repair. Stem cells have unique genome organization and distinct chromatin features such as the bivalent domains. Understanding the spatial regulation of DNA repair fidelity in stem cells is important because stem cells are exposed to high replication stress due to their fast cycling and as they need accurate repair to maintain their identity.

We developed a strategy to simultaneously monitor deletions/insertions formation and knock in efficiency (HR) at DSBs induced by CRISPR/Cas9 at different loci decorated by distinct chromatin marks in mouse Embryonic Stem cells (mESCs) and fibroblasts (3T3 MEFs). Contrary to the common belief that ESCs have increased repair fidelity we find that DSBs in mESCs lead to larger deletions than in differentiated cells. Interestingly, we find that these deletions are polymerase- thetadependent and inhibition of pol-theta leads to increased HR mainly in mESCs and not in differentiated cells. These results suggest that although stem cells are more geared towards HR, they have high levels of MMEJ and the balance between HR and MMEJ is chromatin context dependent. Given the growing importance of genome editing, this work will shed light on the fidelity and precision of genome engineering which is of paramount importance for the implementation of effective and safe gene therapies.

Notes

Mapping DNA damage repair mechanisms in human cells with high-resolution functional genomics

Brittany Adamson
Assistant Professor
Princeton University
Carl Icahn Laboratory
Princeton, US

Human cells use a sophisticated set of DNA repair mechanisms to safeguard their genomes. We develop and use innovative genomics technologies to understand how these mechanisms work at the systems-level—with the particular goal of understanding how response flexibility is achieved across conditions. Recently, we developed a versatile high-throughput approach, called Repair-seq, that measures the effects of thousands of genetic perturbations on mutations introduced at programmed DNA lesions. This platform produces 'high-resolution' phenotypes that, when applied across many genes, can 'map' the DNA repair processes stimulated by the initiating lesions. Using this technique, we have interrogated three genome editing technologies: DNA double-strand break (DSB)-induced editing, base editing, and prime editing. We have also deployed a large-scale genetic interaction mapping technique to interrogate the individual and pairwise loss-of-function effects of perturbating ~500 genes related to DNA repair processes. By measuring these effects in the presence and absence of PARP inhibitors, we have elucidated new gene-gene relationships relevant to how cells respond to this form of genotoxic stress.

Notes

Towards a genetic atlas of human double-strand-break repair

Israel Salguero Corbacho[1], Ernesto López de Alba[1], Daniel Giménez Llorente[2], Ana Losada[2] and **Felipe Cortés-Ledesma[1]**

1 Topology and DNA breaks Group, Spanish National Cancer Research Centre, Madrid, Spain
2 Chromosome Dynamics Group, Spanish National Cancer Research Centre, Madrid, Spain

DNA double-strand breaks (DSBs) are a significant threat to genomic stability, and their accurate repair is crucial for the maintenance of normal cellular function. When DSBs are not repaired correctly, they can lead to chromosomal abnormalities, gene mutations, and ultimately contribute to the development of cancer and other diseases. Besides these detrimental outcomes, programmed DSBs are essential in relevant processes such as the generation or immune diversity and gametogenesis, and constitute the molecular basis for most of CRISPR-Cas9 gene editing approaches.

In order to get a comprehensive insight into DSB repair mechanisms, we have developed and conducted a novel in-pool genome-wide CRISPR-Cas9 genetic screening. Similarly to the recently developed Repair-seq technique, our method relies in molecularly linking each individual repair event to the specific genetic context in which this occurred, as determined by the gRNA expression cassette. Thus, with a single experimental approach we have retrieved molecular information on how each of the more than 17K human genes included in our library affects the outcome of CRISPR-Cas9-induced DSB repair. We will discuss some novel insights into the DSB repair process, as well as implications for CRISPR-Cas9 gene editing approaches.

Notes

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Session #5
THE 3D GENOME II

Chairperson: ***Gaëlle Legube***

Loop-extruding Smc5/6 organizes transcription-induced positive DNA supercoils

Camilla Björkegren

Professor in Cell and Tumour Biology Karolinska Institutet Dept. of Biosciences and Nutrition Dept. of Cell and Molecular Biology, Sweden

The Smc5/6 complex (Smc5/6), initially discovered for its function in DNA repair and recombination, has remained one of the least understood SMC complexes. Recent single molecule- and structural analyses have started to change this, and evidence indicating that Smc5/6 function is connected to DNA supercoiling is accumulating. Here we present results from our investigation of the Saccharomyces cerevisiae complex. Single molecule analysis shows that the Smc5/6 complex performs DNA loop extrusion. This provides a molecular mechanism for the complex and reveals that DNA extrusion is a common activity of all three eukaryotic SMC complexes. Additional investigations indicate that the main determinant for Smc5/6 chromosomal positioning are positive DNA supercoils, which in turn are influenced by gene organization and expression, sister chromatid entanglement, and loop extruding cohesin. Altogether, our results indicate that Smc5/6 uses DNA loop extrusion to organize positively supercoiled regions found at the base of cohesin-dependent chromosome loops.

Notes

SHORT TALK

Genome control by cohesin

Roel Oldenkamp[1], Josh J. Graham[2], Amrita Patel[2], Wessel Rodenburg[1], Klaudia Majszczyk[1], Beccy Harris[1], Yan Li[3], Kyle W. Muir[4], Daniel Panne[2], Benjamin D. Rowland[1]

1 Division of Cell Biology, The Netherlands Cancer Institute, Amsterdam, The Netherlands
2 Leicester Institute of Structural and Chemical Biology, Department of Molecular and Cell Biology, University of Leicester, Leicester, UK
3 Department of Fundamental Microbiology, Faculty of Biology and Medicine, University of Lausanne, Lausanne, Switzerland
4 MRC Laboratory of Molecular Biology, Cambridge, UK

Cohesin shapes the genome by building DNA loops and by connecting the sister chromatids. Cohesin's ability to build CTCF-anchored loops is dependent of CTCF's binding to a conserved essential surface (CES) on cohesin's SA subunits. Using cell biology and structural biology, we reveal two CES ligands that together play an essential role in sister chromatid cohesion. We present our insights into how the interplay between these two CES-binding proteins enables cohesion along chromosome arms.

CES ligands stabilise cohesin on DNA by competing for binding with cohesin's release factor WAPL. We reveal how WAPL competition through a multi-step cohesin binding mode drives DNA release in interphase and mitosis. Based on structural and cellular data, we present a model for how CES-binding factors direct cohesin to structure DNAs. We propose that these factors control the cohesin release cycle by eliminating one specific aspect of WAPL-driven DNA release.

Notes

SHORT TALK

Specific functions and regulation of cohesins STAG1 and STAG2

Ana Losada

Chromosome Dynamics Group Leader Molecular Oncology Programme | Spanish National Cancer Research Centre – CNIO - Madrid, Spain©

Two versions of the cohesin complex carrying the STAG1 or STAG2 paralog are present in all vertebrate cells. Either complex is sufficient to maintain cell viability but mouse embryos require both for proper development. The different chromatin association dynamics of the two cohesins contribute to their functional specificity, although the underlying molecular mechanisms are yet to be defined. STAG2 is among the most recurrently mutated genes in several types of cancer, one of them being Ewing sarcoma. Mutations in *NIPBL*, which cause Cornelia de Lange Syndrome, likely affect differentially the two cohesins. Why cells need two different cohesins and what happens when one of them is malfunctioning are relevant questions to understand disease. I will discuss our recent progress in addressing them.

Notes

Mechanisms of genome "awakening" and 3D chromatin reorganization at the start of life

Kikue Tachibana
Max Planck Institute of Biochemistry, Martinsried, Germany

How chromatin is reprogrammed to a totipotent "ground state" at the start of life remains a crucial question in biology. Totipotency is the developmental potential of a cell to give rise to all cell types and a whole organism. Reprogramming in early embryos is thought to occur by resetting of epigenetic modifications and activation of embryonic transcription in a process known as zygotic genome activation (ZGA). Despite the fundamental importance of ZGA for subsequent development, its essential regulators remain largely unknown in mammals. We recently identified the orphan nuclear receptor Nr5a2 as an essential transcription factor that activates the majority of ZGA genes in mouse 2-cell embryos. Genomic and biochemical data provide evidence that Nr5a2 has pioneering activity to open chromatin. We conclude that Nr5a2 is a key pioneer factor that transcriptionally "awakens" the genome during ZGA.
Early development is also accompanied by changes in genome architecture. There is mounting evidence that a mechanism of loop extrusion folds the genome into loops and topologically associating domains (TADs). Loop extrusion is halted at genomic sites bound by the zinc finger transcription factor CTCF, which is the main barrier to loop extrusion. By taking advantage of the oocyte-to-embryo transition, we discovered that the minichromosome maintenance complexes (MCMs) that function as replicative helicases during DNA replication impede loop extrusion in G1 phase. Therefore, 3D genome organization is shaped by loop extrusion and distinct types of barriers.

Notes

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Session #6
THE GENOME IN HEALTH AND DISEASE II

Chairperson: ***Felipe Cortés***

Topoisomerases, Chromatin Architecture & Neural Genome Instability

L.C. Dumitrache, S.M. Downing, R. Henry, H.R. Russell & **P.J. McKinnon**

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Topoisomerase activity critically regulates torsional stress in DNA during replication, chromatin remodeling and transcription. Aberrant topoisomerase function has been implicated in diverse neurological disease including autism, spinocerebellar ataxias such as ataxia telangiectasia and neurodevelopmental disorders. However, the etiologic impact of topoisomerase dysfunction and how this underpins the pathogenesis of neurologic disease is unclear, making, understanding topoisomerase biology of paramount importance. While Topoisomerases are critical for cellular function, inactivation of their activity often renders a cell non-viable, making physiologic evaluation of their tissue-specific roles problematic. To determine the pathobiology of topoisomerase dysfunction we generated murine models of TOP2b with an endogenous "trapping" point mutation, which impedes catalytic activity and promotes DNA damage. Neural expression of the TOP2b mutation increased toxic TOP2-cleavage complexes leading to specific DNA damage-induced perturbations of neurogenesis. Remarkably, endogenous TOP2b-induced DNA damage was restricted to select neural cell populations, including cerebellar Purkinje cells and cortical oligodendrocytes. This phenotype was markedly exacerbated by TDP2 or ATM inactivation. Notably, Purkinje cells are selectively affected when various DNA repair strand break pathways are disabled throughout the nervous system where unrestrained DNA breaks lead to transcriptional interference in the cerebellum via aberrant messenger RNA splicing, disrupting the expression of critical homeostatic regulators including ITPR1, GRID2, and CA8. Using ATAC-seq to profile global chromatin accessibility in the cerebellum, we found a unique chromatin conformation specifically in Purkinje chromatin at affected gene loci, thereby promoting susceptibility to DNA damage. These data suggest chromatin conformation is a feature in directing genome instability-associated neuropathology after endogenous DNA damage such as aberrant Topoisomerase function.

Notes

PARP1 protein in DNA Repair and Human disease

Keith W. Caldecott, PhD FMedSci
Deputy Director, Genome Damage and Stability Centre, University of Sussex, Brighton, UK

PARP1 and/or PARP2 promote the repair of DNA strand breaks via a number of molecular mechanisms, including the remodelling of chromatin and the direct recruitment of DNA repair enzymes. In diseases in which DNA repair is attenuated however, PARP1 signalling can result in cellular toxicity and/or pathogenicity. Here, I will describe new work identifying novel mechanism/s by which PARP activity protects cells during normal cellular proliferation, and/or by which aberrant ADP-ribosylation can impact on genome integrity and cell function. These data shed new light on both the positive and negative impacts of PARP activity on genome integrity, genome function, and disease pathology following DNA damage.

Notes

SHORT TALK

Deciphering the mechanism of toxicity by nucleolar stress

Oscar Fernandez-Capetillo
Molecular Oncology Programme Director. Genomic Instability Group Leader
Spanish National Cancer Research Centre – CNIO Madrid, Spain

Nucleolar stress (NS) is a loosely defined term that encompasses a wide range of insults targeting nucleoli. While associates to several diseases such as cancer or neurodegeneration, a full understanding of how NS leads to cell death is lacking. Early works indicated that NS triggers P53-dependent apoptosis through the binding of ribosomal proteins to MDM2. However, if NS persists, P53-deficient cells also die from this insult, indicating that other mechanism must underly the general toxic effects associated to it. Using as a model a toxic arginine-rich peptide found in patients of ALS, which has particular impact on nucleoli, we have performed multiple experiments trying to understand the basis of this phenomenon. Our data converge on a model where damage to the nucleoli invariably leads to an accumulation of free ribosomal proteins in the cytoplasm, also known as a ribosomopathy. Given that ribosomal proteins are the most abundant in the proteome, their accumulation is known to saturate proteasomal pathways or chaperones. We are confident that our work provides a general model to understand how NS impairs cell fitness in mammalian cells.

Notes

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MEETINGS

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Organisers & Speakers' Biographies

cnio stop cancer

Felipe Cortés

Topology and DNA breaks Group Leader Molecular Oncology Programme
Spanish National Cancer Research Centre – CNIO, Madrid, Spain

Felipe Cortés-Ledesma (born in Sevilla in 1977) Graduated in Biology in the University of Seville, and obtained his PhD in Molecular Biology from the same University under the guidance of Prof. Andrés Aguilera on the molecular mechanisms of DNA break repair using yeast as a model organism. He then joined the laboratory of Prof. Keith Caldecott at the Genome Damage and Stability Centre (University of Sussex, UK) to expand his training on DNA breaks and the DNA-damage response into mammalian systems.

After three years, he obtained a Ramón y Cajal Fellowship, with which he established himself independently at the Andalusian Centre of Molecular Biology and Regenerative Medicine (CABIMER), where, shortly after, he obtained a permanent position as Científico Titular, and more recently, a promotion to Investigador Científico in the Spanish National Research Council (CSIC).

In 2019 he joined the Spanish National Cancer Research Centre (CNIO) leading the Topology and DNA breaks Group, where he is continuing his research line on how DNA topology shapes the organization and dynamics of the genome, and how an imbalance in these processes can lead to the appearance of pathological DNA breaks that compromise genome stability.

In summary, Felipe Cortés-Ledesma has devoted his scientific career to the study of DNA breaks, and in particular to those related with the topology of the DNA molecule, a field in which he has made seminal contributions. Important achievements that can be highlighted are publications as senior author in top journals, such as Nature and Science, the election as EMBO Young Investigator, and the award of an ERC Consolidator Grant.

Oscar Fernandez-Capetillo

Molecular Oncology Programme Director Genomic Instability Group Leader
Spanish National Cancer Research Centre – CNIO, Madrid, Spain

Oscar Fernandez-Capetillo joined CNIO in 2005 to lead the Genomic Instability Group where he has been ever since. Initial works from the lab concentrated on exploring the role of replicative stress in cancer and ageing, for which the group combined cell biology, mouse models and drug development projects. More recently, the group has expanded to other areas such as mechanisms of drug resistance and neurodegenerative diseases. Since 2015 Oscar is also Vicedirector of CNIO and the Director of its Molecular Oncology Programme, as well as a professor at the Karolinska Institute in Sweden.

Ana Losada

Chromosome Dynamics Group Leader Molecular Oncology Programme |
Spanish National Cancer Research Centre – CNIO, Spain

Ana Losada obtained her PhD in Biochemistry from Universidad Autónoma de Madrid after her work on centromere specification in flies. She then joined Tatsuya Hirano's group at Cold Spring Harbor Laboratory (USA) where she identified and characterized the first vertebrate cohesin complex. In 2004, she started her own group at the CNIO where she has continued to explore how cohesin works and how its dysfunction contributes to cancer and other human diseases. She is particularly interested in the specific functions of variant cohesin complexes STAG1 and STAG2 in genome inheritance and 3D genome folding.

Andre Nussenzweig

Laboratory of Genome Integrity (LGI), National Cancer Institute (NCI)
National Institutes of Health – NIH, US

Andre Nussenzweig received his Ph.D. in Physics from Yale University in 1989. Subsequently, Dr. Nussenzweig became a Research Fellow at Memorial Sloan-Kettering Cancer Center prior to joining the Experimental Immunology Branch as a tenure track investigator in 1998. Dr. Nussenzweig received tenure at NIH in 2003. In 2011, Dr. Nussenzweig established a new department at NCI called the Laboratory of Genome Integrity. Dr. Nussenzweig is an elected member of the European Molecular Biology Organization, a National Institutes of Health Distinguished Investigator, a 2019 inductee into the US National Academy of Medicine and a 2021 Basser Global Prize winner for BRCA cancer research.

Brittany Adamson

Assistant Professor Princeton University Carl Icahn Laboratory Princeton, US
https://www.badamsonlab.com/britt

Brittany Adamson is an assistant professor in the Department of Molecular Biology and the Lewis-Sigler Institute for Integrative Genomics at Princeton University. She is also a member of the Genomic Instability and Cancer Genetics Program at the Rutgers Cancer Institute of New Jersey. Dr. Adamson started her training in 2004 at the Massachusetts Institute of Technology in the laboratory of Angelika Amon. In 2007, she joined the lab of Stephen Elledge at Harvard Medical School, where she used functional genomics approaches to study DNA repair in human cells. She earned her PhD from Harvard Medical School in 2012. Dr. Adamson then worked with Jonathan Weissman at the University of California, San Francisco, where she received a postdoctoral fellowship from the Damon Runyon Cancer Research Foundation. Her postdoctoral work pioneered new approaches for functional genomics in human cells, technologies that now enable dissection of cellular pathways with unprecedented resolution. With her lab at Princeton, Dr. Adamson continues to use and develop cutting-edge experimental tools, including genetic screening methods and single-cell RNA-sequencing, to study genome editing and DNA repair, as well as other areas of interest to the group. Dr. Adamson is the recipient of a 2020 Searle Scholars Award and Rutgers Cancer Institute of New Jersey New Investigator Award.

Frederick Wayne Alt

Harvard Medical School, US

Frederick Alt is a Howard Hughes Medical Institute Investigator and Director of the Program in Cellular and Molecular Medicine at Boston Children's Hospital. He is the Charles A. Janeway Professor of Pediatrics and Genetics at Harvard Medical School. He received his Ph.D. from Stanford University where he discovered gene amplification in mammalian cancer cells. His past work has contributed to elucidating mechanisms of programmed rearrangements in lymphocytes and pathways and mechanisms of end-joining repair of DNA double strand breaks. His current research focuses on elucidating fundamental mechanisms of antigen receptor genes diversification and developing mouse models for testing HIV-1 vaccine strategies and for discovering humanized antibodies against emerging pathogens.

Luis Aragón

DNA Motors
MRC London Institute of Medical Sciences - LONDON, UK

Luis Aragón is the Head of the DNA motors Group at the MRC London Institute of Medical Sciences (LMS) in London. He established his group at the MRC LMS in 2002. His laboratory is focused on the function of the three eukaryotic (SMC) complexes; cohesin, condensin and Smc5/6. Currently, the Aragon lab uses biochemical and biophysical techniques on purified proteins to investigate mechanisms of SMC complex function, and combine these *in vitro* approaches with studies in yeast and human cells to gain an understanding of the function of these protein complexes.

photographer A. Donka

Camilla Björkegren

Professor in Cell and Tumour Biology - Karolinska Institutet - Dept. of Biosciences and Nutrition
Dept. of Cell and Molecular Biology - Sweden

Camilla Björkegren is professor at the Department of Cell and Molecular Biology, Karolinska Institutet, Stockholm, Sweden where her research team explores molecular mechanisms behind chromosome organization and function. She started her career at Stockholm University, obtained a PhD degree in 1997, and was a postdoctoral fellow at the Institute of Molecular Pathology (IMP), Vienna, Austria until 2001. With her move to IMP, she changed field of research and initiated her current line of investigation which focuses on the family of Structural Maintenance of Chromosome protein complexes.

Maria A. Blasco

Scientific Director, CNIO
Head, Telomeres and Telomerase Group (CNIO)
Spanish National Cancer
Research Centre, CNIO

Maria A. Blasco is a molecular biologist devoted to the study of telomeres and telomerase and their role in cancer and aging, a field of research in which she excels worldwide. Her work has been published in high impact journals as, Cell, Nature or Science, among others.

Blasco has merited vast recognition, both national and international, as the Joseph Steiner Award of Switzerland, the EMBO Gold Medal, the Körber European Science Award of Germany, as well as the Spanish National Research Award in Biology Santiago Ramón y Cajal (2010) and the Premio Jaume I, among others.

Blasco was named Chair of SOMMa ('Severo Ochoa' Centres and 'María de Maeztu' Units of Excellence Alliance) and has received three Doctorate Honoris Causa in Spain: Universidad Carlos III of Madrid (2014), Universidad of Alicante (2017) and Universidad of Murcia (2018). In October 2017 received the Generalitat Valenciana Scientific Award.

Keith W. Caldecott

Deputy Director, Genome Damage and Stability Centre,
University of Sussex, Brighton, UK

Keith Caldecott became interested in how cells repair DNA strand breaks during his PhD with Penny Jeggo at the National Institute for Medical Research, and his interest in DNA strand-break repair evolved further during postdoctoral positions in with Larry Thompson in California and Tomas Lindahl in London. Keith established his own laboratory at the University of Manchester in 1995 and relocated to the Genome Damage and Stability Centre in Sussex, in 2002. Keith's research is focussed on identifying novel human genes involved in DNA strand-break repair and uncovering their roles in preventing human genetic disease.

Victor G. Corces
Professor. Department of Human Genetics
Emory University School of Medicine - Atlanta, US

Victor Corces received his PhD from the Complutense University of Madrid and was a postdoctoral Fellow in the Department of Biochemistry and Molecular Biology at Harvard University, where he worked with Matt Meselson. He was Assistant/Associate/Professor/Chair in the Department of Biology at Johns Hopkins University. He moved to Emory University in 2007 where he is currently a Professor of Human Genetics at the Emory University School of Medicine. Research in Dr. Corces' laboratory studies the mechanisms by which the environment alters the epigenome of germline cells and how these epigenetic alterations can be transmitted from the gametes to the embryo after fertilization to cause disease states that can be passed on to subsequent generations. He has published extensively in the area of epigenetics and genomics, with a focus on the 3D organization of the nucleus of egg and sperm and the mechanisms of transgenerational inheritance of epiphenotypes.

Gaëlle Legube

Chromatin and DNA repair group Molecular, Cellular & Developmental Biology Unit
CBI (Centre De Biologie Integrative)
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After her undergraduate studies in Poitiers and Lyon in France, Gaëlle Legube performed her PhD in the group of Didier Trouche in Toulouse on the histone acetyltransferase Tip60 in human cells. She further joined as a postdoctoral fellow the group of Asifa Akhtar at EMBL to work on dosage compensation in Drosophila. There, she started to use genome wide approaches, reporting the first high resolution profile of the dosage compensation complex on the chromosome X using ChIP-chip.

After obtaining a permanent position at the CNRS in France, she started her own lab at the CBI, in Toulouse in 2011. Her lab focuses on the function of chromatin during DNA Double Strand Break Repair. In 2019, Gaëlle was awarded several prizes, such as the bronze and silver medals from the CNRS, the Coup d'Elan from the Fondation Bettencourt and the prix Raymond Rosen from the Fondation pour la recherche medicale.

Peter J. McKinnon

Member, St. Jude Faculty Vice-Chair, Department of Cell & Molecular Biology
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Peter McKinnon received his PhD from Adelaide, Australia where he worked on chromatin structure and ataxia telangiectasia (A-T), subsequently developing an interest in the neurobiology of A-T. His postdoctoral work focused on molecular neurobiology and mouse genetics. Upon moving to St. Jude Children's Hospital as a faculty member, he established a research program to study the neurobiology of A-T. This work broadened to examine the relationship between DNA damage signaling, genome instability and disease in the nervous system. He is the Director for the Center for Pediatric Neurologic Disease Research and Vice-chair of the Department of Cell and Molecular Biology.

Jan-Michael Peters

Scientific Director, Mitosis and chromosome biology
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Jan-Michael Peters, born in Germany, studied biology in Kiel and Heidelberg. He obtained his Ph.D from the University of Heidelberg in 1991 where he worked with Werner Franke, discovered p97-ATPase and the AAA-ATPase family, and first purified and structurally characterized the 26S proteasome. As a postdoctoral fellow with Marc Kirschner at Harvard Medical School in Boston he discovered the anaphase promoting complex/cyclosome (APC/C) and other enzymes required for chromosome segregation. In 1996 he became Junior Group Leader, in 2002 Senior Scientist, in 2011 Scientific Deputy Director and in 2013 Scientific Director of the Institute of Molecular Pathology (IMP) in Vienna. His lab is studying genome architecture and chromosome segregation in mammalian cells and made important contributions to understanding the molecular mechanisms of these processes, including the hypothesis that cohesin and CTCF contribute to genome architecture by forming chromatin loops. He is an author on more than 180 publications, has received a number of awards including the Wittgenstein Prize and two ERC advanced grants and has coordinated several large-scale research projects, such as the European Union projects MitoCheck and MitoSys.

Ana Pombo

Berlin Institute for Medical Systems Biology at the Max Delbrück Centre for Molecular Medicine, and Humboldt University of Berlin, Berlin, Germany

Ana Pombo studies mechanisms involved in 3D genome folding and gene expression during mammalian development and in disease. After her DPhil at University of Oxford, UK, Ana was the recipient of Royal Society Dorothy Hodgkin Fellowship and started her independent lab in London at the MRC London Institute for Medical Sciences. In 2013, she moved her laboratory to the Berlin Institute of Medical Systems Biology (BIMSB) of the Max Delbrück Center in Berlin, Germany, and was co-appointed Professor at the Humboldt University of Berlin. Ana currently serves as Deputy Director of BIMSB. Ana is a recipient of the Robert Feulgen Prize, and is elected member of EMBO and the European Academy of Sciences. She is member of the NIH 4D-Nucleome consortium, and has pioneered the development of Genome Architecture Mapping (GAM), an orthogonal technology to map the 3D structure of chromosomes genome-wide.

Marie-Noëlle Prioleau

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M-N Prioleau, director of research at Inserm, leads a team at the Jacques Monod Institute (IJM, Paris, France). She obtained her PhD in Molecular biology in 1994, working with Dr. M. Méchali. Then, M-N Prioleau stayed for three years in the laboratory of Dr. G. Felsenfeld at the NIH (Bethesda, USA). In 1998, she joined the team of O. Hyrien at the ENS in Paris where she started to work on mechanisms involved in origin selection. In 2005, she joined the IJM to establish her laboratory. Her group was the first to map replication origins at a large scale in human cells, to explore the link between replication origins and epigenetic marks, to demonstrate the role of G-quadruplex (pG4) in the activity of model origins and finally to identify dimeric pG4s as key organizers of replication origins in vertebrates.

Vassilis Roukos

Assistant Professor of Biology at the Department of Medicine of the University of Patras, Greece
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Dr. Vassilis Roukos is an Assistant Professor of Biology at the Department of Medicine of the University of Patras, Greece and affiliated Group Leader at the Institute of Molecular Biology (IMB), Mainz, Germany. He holds a PhD degree in Molecular Biology and Cytogenetics from the Department of Medicine in Patras, Greece and has served as a Postdoctoral Researcher and Research Fellow at the NCI of the NIH, USA. From 2015 onwards he is leading a research group focusing on cellular and molecular pathways counteracting genomic instability established at IMB in Germany, which was recently relocated to Patras, Greece.

Kikue Tachibana
Max Planck Institute of Biochemistry
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Kikue Tachibana studied Natural Sciences at Cambridge University and worked on DNA replication in the laboratory of Ron Laskey at the MRC Cancer Cell Unit for her PhD. Kikue joined the laboratory of Kim Nasmyth at Oxford University to investigate sister chromatid cohesion in mouse oocytes. She started her own research program on zygotic reprogramming as a junior group leader at the Institute of Molecular Biotechnology, Vienna. In 2019 Kikue joined the Max Planck Institute of Biochemistry, Munich, as Director of the Department of Totipotency. Kikue is an EMBO member and was honored with the Walther Fleming Award and the City of Vienna Prize.

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Genome organisation and stability

Poster Session

cnio stop cancer

1

DNA Damage Overcomes Tumor Immune Infiltration Deficiency in Colorectal Cancer

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Colorectal cancer (CRC) is a complex heterogeneous disease with varying gene expression profiles, impacting prognosis and therapeutic response. Unfortunately, only a small percentage of CRC cases exhibit significant tumor immune infiltration, which limits treatment options with immune checkpoint blockades (ICBs). Using genetically engineered mouse models of CRC, we show that the loss of the molecular cochaperone unconventional prefoldin RPB5 interactor (URI) increases intra-tumoral DNA damage via defects in NHEJ repair, consequently, enhancing DNA sensing receptor pathways and immune cell infiltration in the tumor.

Furthermore, bioinformatics analysis of publicly available human datasets revealed that URI expression significantly correlates with tumor mutational burden (TMB), tumor-infiltrating immune cell profiles, and anti-PD1 therapeutic response in human cancers. Our findings define URI as a potential biomarker and neoadjuvant target for ICB in CRC and hence, highlight the importance of inhibiting URI to increase DNA damage and TMB to sensitize CRC to immunotherapy and improve patient outcomes.

Tyrosyl-DNA phosphodiesterase 2 (TDP2) prevents genome instability associated with TOP2b catalytic inhibition

2

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DNA topoisomerase II (TOP2) enzymes, namely TOP2A and TOP2B, play a crucial role in regulating genome dynamics by resolving complex 3D topological structures that arise from DNA metabolism. Different classes of drugs can target the TOP2 catalytic cycle, including TOP2 poisons and TOP2 inhibitors. While TOP2 poisons have been extensively studied due to their use in chemotherapy, the cellular responses to the formation of TOP2 closed clamps by TOP2 inhibitors remain less understood. Through a CRISPR dropout screening, we identified that the DNA repair enzyme tyrosyl-DNA phosphodiesterase 2 (TDP2), which removes trapped TOP2 cleavage complexes (TOP2cc) formed by TOP2 poisons, also affect the cellular response to the TOP2 inhibitor ICRF-187. Deletion of TDP2 in human RPE-1 cells increases sensitivity to low concentrations of ICRF-187, resulting in failure of late replication completion and consequent G2 cell cycle arrest. This arrest is dependent on the TOP2B isoform, which is the preferential target of ICRF-187, but occurs without TOP2cc accumulation and independently of TDP2 catalytic activity. The arrest is mediated by the ATR and Chk1 checkpoints sensor kinases, but interestingly, independent of the accumulation of DNA breaks. ATR inhibition can bypass the G2 arrest, allowing TDP2-deleted cells to progress through the cell cycle, but this comes at the cost of accumulating mitotic aberrations and micronuclei in daughter cells. In summary, this study highlights the replication-associated TOP2B closed clamps as an important source of genome instability and uncovers a role of TDP2 in their resolution in a phosphodiesterase-independent manner.

3

A functional link between separase and Sak1 in mitosis

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A crucial process during mitosis is the correct segregation of genetic material into the two new daughter cells. This process, which is highly regulated and controlled, is initiated in eukaryotic cells by the caspase-like protein separase (Esp1). Separase cleaves the Scc1 subunit of the cohesin complex allowing the segregation of the sister chromatids in anaphase. Separase, in addition to the main function in chromosome segregation, it is also involved in promoting mitotic spindle elongation, Cdc14 activation and rDNA segregation. The direct substrates of separase in these processes are still unknown. Through a genetic screen of separase suppressor genes, we discovered a functional link in mitosis between separase and the protein Sak1. Nowadays, it is established that Sak1 is an activator of the kinase Snf1, but our results show that Sak1 promotes Esp1-regulated functions in mitosis independently of the Snf1 pathway. Additionally, Sak1 exhibits synthetic lethal interactions with MEN mutants, suggesting that it may be a new component of the FEAR pathway, regulating the activation of Cdc14 during anaphase. Our results suggest a role for Sak1 as a new mitotic regulator, through the phosphorylation of separase.

Coordination of the chromosome integrity functions of BRCA2 in mitosis

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Chromosome integrity entails the correct alignment and segregation of chromosomes into daughter cells. Defects in this process lead to aneuploidy, a hallmark of cancer. Recent work from our team revealed that the phosphorylation of BRCA2 at its N-terminus by PLK1 allows a distinct role of BRCA2 in chromosome alignment (Ehlen et al., Nat.Commun., 2020). In this context, BRCA2 is in complex with key players ensuring stable kinetochore-microtubules attachments such as PLK1, BUBR1, and PP2A. When this complex is impaired, faulty chromosome alignment/segregation follows. To better understand how these complexes are coordinated at the kinetochore (KT) we investigated a specific region of BRCA21093-1158 between BRC repeats 1 and 2 reported to bind PP2A (Hertz et al., Mol. Cell, 2016) which harbors phosphosites for PLK1 (Julien et al., Biomolecules 2021). We identified S1115 and S1121 as key PLK1-phosphosites in mitosis. Their bi-phosphorylation favors the interaction of BRCA2:PP2A *in vitro*. We selected breast cancer variants S1115C and S1121L and showed that one maintains the interaction with PP2A whereas the other reduce it *in vitro* and set out to study their phenotype in cells. Cells bearing BRCA2-S1121L showed severe chromosome misalignment and reduced BRCA2 binding to PLK1 and PP2A suggesting that BRCA21093-1158 interaction with PP2A and/or PLK1 is important for chromosome alignment. We hypothesize that BRCA2 might be orchestrating the recruitment of PLK1 and PP2A to the KT to ensure KT-microtubules stability. Investigating how these BRCA2 complexes are modulated in and outside of mitosis in cells bearing these variants, we expect to get insights into the coordination of DNA repair and chromosome segregation processes in the cell. This work might provide clues on the mechanisms of chromosome instability found in breast cancer patients bearing these variants.

5

Molecular architecture and oligomerization of *C. glabrata* Cdc13 dictates its binding to telomeric DNA

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The CST complex, composed of Cdc13, Stn1 and Ten1 in yeast, mediates the replication and stability of telomeric DNA. Cdc13, the least evolutionarily conserved component, features four concatenated OB-fold domains, whose architecture and functions remain poorly understood. We dissected the molecular architecture of *Candida glabrata* Cdc13 and showed how each of its OB folds contributes to its self-association and binding to telomeric DNA sequences. Using a combination of biochemical and biophysical tools, we concluded that all individual domains contribute to DNA binding despite not being directly implicated in the binding itself. Analyzing Cdc13 mutants lacking one or more OB-fold domains, we observed that Cdc13 forms dimers primarily through the interaction between OBfold 2 (OB2) domains, stimulating the binding of OB3 to telomeric sequences.

Furthermore, we showed that *C. glabrata* Cdc13 and CST form higher-order complexes via oligomerization through OB1. Truncated forms of Cdc13, lacking the OB1 domain, abolish oligomerization and result in shortening of telomeres *in vivo.*

Our results reveal the molecular organization of *C. glabrata* Cdc13, its relationship with DNA binding and telomere elongation, and imply that the distinct architectures of yeast Cdc13 share common principles

URI loss-induced DNA damage response serves as a barrier against colorectal cancer

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Colorectal cancer (CRC) is the second leading cause of cancer-related deaths worldwide. It is a multi-hit neoplasia that usually evolves from APC mutationinduced adenomatous polyps that become malignant by acquiring p53 loss. Yet the mechanisms of the transitional mutations for the development of aggressive tumours remain to be elucidated. Here we show that the oncogenic protein URI (Unconventional Prefoldin RPB5-Interactor) is upregulated in human CRC tumors at both protein and mRNA levels, and its expression correlates with a further progression of the disease, especially in patients harbouring APC mutations and Wnt pathway activation. Using genetically engineered modified mouse models, we demonstrate that reducing URI levels in the intestine prevents CRC progression in different intestinal cancer models in a p53-dependent manner. Mechanistically, URI downregulation impairs the non-homologous end joining (NHEJ) mechanism for DNA damage repair, leading to a p53-mediated DNA damage response.

Furthermore, URI directly interacts with p53 to facilitate its degradation, a mechanism that also contributes to p53 stabilization upon URI loss, establishing a DNA damage response barrier that prevents CRC progression. Our data reveal a previously unrecognized mechanism to control p53 levels relevant for the progression of CRC.

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Exploring new regulators of R-loop-mediated telomere stability

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DNA-RNA hybrid structures known as R loops are transcription byproducts that not only play physiological regulatory roles in specific situations but also pose a major threat to genome stability. R-loops can hamper replication fork progression leading to hyperrecombination and genome instability. R-loop regulation is also crucial at telomeres, having thus a potential role in human health provided that telomere stability is essential to prevent tumorigenesis and cellular senescence in human cells. Telomeres are transcribed into TERRAs, which have the ability to form R-loops and indeed, R-loops are accumulated at telomeres of cancer cells that use the Alternative Lengthening of Telomere (ALT) maintenance mechanism to gain immortalisation, for which they have been proposed as drivers of ALT recombination. The mammalian ATR-mediated replication stress signalling pathway, the RAD18-UBE2B ubiquitin-ligase complex involved in post-replicative repair, the RNA helicase UAP56 and other RNA-binding factors have been recently uncovered as main regulators of R-loop accumulation, suggesting that DNA-RNA hybrids are differentialy processed along the cell cycle (Barroso, EMBO Rep 2019; Perez- Calero, Genes Dev, 2020). Interestingly, despite the fact that TERRA levels vary in a cell cycle-dependent manner in telomerase-positive cells, whether telomeric DNARNA hybrids are differentialy regulated through the cell cycle is unknown. We are currently investigating the molecular mechanisms that control R-loop modulation and R-loop-associated instability of human telomeres by focussing on their regulation by replicave and post-replicave DNA damage responses and specific RNA-processing factors know to control R-loops genome wide.

Improved cohesin HiChIP protocol and bioinformatic analysis for robust detection of chromatin loops and stripes in human cells

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Chromosome Conformation Capture (3C) methods, including Hi-C ("all-versus-all" variation of 3C), detect pairwise interactions between DNA regions, enabling the reconstruction of chromatin architecture in the nucleus. HiChIP is a modification of the Hi-C experiment, which includes a chromatin immunoprecipitation step (ChIP), allowing genome-wide identification of chromatin contacts mediated by a protein of interest. In mammalian cells, protein complex cohesin, together with CCTCbinding factor (CTCF), are major players involved in the establishment of chromatin loops. Here we present an improved HiChIP protocol and bioinformatic analysis approach that includes a pipeline for automatized processing of multiple samples.

When cohesin HiChIP was performed using two cross-linking agents (formaldehyde [FA] and EGS) instead of typically used FA alone, a significantly better signal-tonoise ratio and a higher ChIP efficiency were observed. This led to the detection of a significantly higher number of chromatin loops and a better loop enrichment score, as assessed by two independent loop calling algorithms. Moreover, we report a novel tool called gStripe for detecting architectural stripes - patterns of frequent interactions between a loop anchor and a continuous range of loci, considered to emerge as the result of the one-sided loop extrusion process.

gStripe is designed to detect stripes based on the topology of the interaction networks. It performed better for most of the cohesin and CTCF HiChIP datasets analysed as compared to the image processing-based method, StripeNN. Finally, we propose a new loop extrusion modelling (LEM) approach, which uses CTCF motif positions and orientation within HiChIP peaks, as well as biophysical knowledge of the motion of cohesin, to generate dynamic 3D models of loop extrusion for a region of interest.

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The phosphatase-2A subunit PR130/PPP2R3A controls checkpoint kinases, cell fate, and DNA repair pathway choice upon DNA replication stress

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Background
Replicative stress can lead to genomic instability and cellular transformation. The phosphorylation-dependent activation of the checkpoint kinases ATM, ATR, CHK1/CHK2, and WEE1 slows down the cell cycle and promotes DNA repair. Epigenetic modulators of the histone deacetylase (HDAC) family affect checkpoint kinase signaling. Ongoing research strives to define the molecular interactions of such proteins. We hypothesized that HDACs modulate checkpoint kinase signaling.
Results and Conclusions
We show that class I HDACs sustain the phosphorylation-dependent activation of checkpoint kinases and the tumor suppressor p53. Transcriptomics suggested that this is linked to the expression of PPP2R3A/PR130, a regulatory subunit of the trimeric serine/threonine phosphatase 2 (PP2A) complex. We could show that specifically HDAC1 and HDAC2 suppress the mRNA and protein expression of PR130. Moreover, pharmacological inhibition of HDAC1, HDAC2, and HDAC3, triggers acetylation of PR130. Using CRISPR-Cas9, we could reveal that PR130 is a novel regulator of the phosphorylation of ATM, CHK1, and p53 in cells with stalled and collapsed DNA replication forks. Ablation of PR130 slows G1/S phase transition and increases phosphorylated CHK1, replication protein A foci, and DNA damage. Accordingly, stressed PR130 null cells are very susceptible to class I HDAC inhibition, which abrogates the S phase checkpoint, induces apoptosis, and reduces the homologous recombination protein RAD5. Nonetheless, HDACi do not increase genomic instability of cells surviving DNA replication stress.
Phosphoproteomics additionally disclosed that the cell cycle regulators p21 and WEE1 are targets of PP2A-PR130. Genetic strategies and specific inhibitors suggest that PR130 controls cell cycle arrest, DNA repair pathway choice, and cell fate upon DNA replication stress through p21 and WEE1. Thus, acetylation-dependent control of PR130 controls cell fate upon DNA replication stress.

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Mechanisms of resistance to anticancer therapies

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Resistance to cancer therapy is a major medical problem, affecting 90% of patients with metastatic cancer. While many patients show variable degrees of response to the initial treatment, advanced disease frequently present resistance to multiple therapies. Unfortunately, many of the mechanisms underlying this phenomenon remain unknown. By combining forward genome-wide CRISPR-screens and transcriptomics, our goal is to discover new mechanisms of resistance which can help us predict future issues in the clinical use of the therapies, as well as identify strategies to overcome such resistance. Our initial screens using novel therapies such as inhibitors of the methyltransferase SETD8 or the USP7 deubiquitinase have allowed us to identify new mechanism that might confer resistance to cancer cells to chemotherapy, and which include modulation of ribosome biogenesis, MYC or tyrosine kinase signaling. We are currently validating the relevance of these observations in several models. Interestingly, part of this work has allowed us to make interesting observations related to the potential senolytic activity of various drugs. Our findings and progress in these areas will be presented.

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Emetine blocks DNA replication via proteosynthesis inhibition not by targeting Okazaki fragments

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DNA synthesis of the leading and lagging strands works independently and cells tolerate single-stranded DNA generated during strand uncoupling if it is protected by RPA molecules. Natural alkaloid emetine is used as a specific inhibitor of lagging strand synthesis, uncoupling leading and lagging strand replication. Here, by analysis of lagging strand synthesis inhibitors, we show that despite emetine completely inhibiting DNA replication: it does not induce the generation of single-stranded DNA and chromatin-bound RPA32 (CB-RPA32). In line with this, emetine does not activate the replication checkpoint nor DNA damage response. Emetine is also an inhibitor of proteosynthesis and ongoing proteosynthesis is essential for the accurate replication of DNA. Mechanistically, we demonstrate that the acute block of proteosynthesis by emetine temporally precedes its effects on DNA replication.
Thus, our results are consistent with the hypothesis that emetine affects DNA replication by proteosynthesis inhibition. Emetine and mild POLA1 inhibition prevent S-phase poly(ADP-ribosyl)ation. Collectively, our study reveals that emetine is not a specific lagging strand synthesis inhibitor with implications for its use in molecular biology.

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Exploring mechanisms of PARPi-induced replication fork acceleration

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Poly (ADP-ribose) polymerase inhibitors (PARPi) are a promising class of anticancer drugs targeting multiple cellular processes including DNA repair and DNA replication. It was previously thought that PARPis induce replication fork stalling and collapse leading to DNA double-strand breaks and eventually cell death in cells with defective homologous recombination repair and/or replication fork protection pathways. We recently confronted this model with the observation that PARPis do not cause fork stalling and rather induce replication fork acceleration. Considering the importance of DNA replication in human diseases, little is known about molecular mechanisms of PARPi-induced replication fork acceleration.

The primary cause of PARPi-induced replication fork acceleration could be the higher velocity of replication forks or reduced origin density since these two processes influence each other. To identify the primary cause of PARPi-induced fork acceleration we combined PARPi treatment with the aphidicolin that targets replication fork rate and observed restoration of origin density, indicating that reduced origin activity in response to PARPi is a secondary response to accelerated forks.

Following these findings, we performed a DNA combing combined with a smallscale custom-designed siRNA library targeting all human DNA polymerases to identify polymerases responsible for PARPi-induced replication fork acceleration.

We are now validating the data from the screen and our results will be discussed at the symposium.

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Disruption of NIPBL/Scc2 in Cornelia de Lange Syndrome provokes cohesin genome-wide redistribution with an impact in the transcriptome

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Cornelia de Lange syndrome (CdLS) is a rare disease affecting multiple organs and systems during development. Mutations in the cohesin loader, NIPBL/Scc2, were first described and are the most frequent in clinically diagnosed CdLS patients. The molecular mechanisms driving CdLS phenotypes are not understood. In addition to its canonical role in sister chromatid cohesion, cohesin is implicated in the spatial organization of the genome.

We investigated the transcriptome of CdLS patient-derived primary fibroblasts and observed the downregulation of genes involved in development and system skeletal organization, providing a link to the developmental alterations and limb abnormalities characteristic of CdLS patients.

Genome-wide distribution studies demonstrate a global reduction of NIPBL at the NIPBL associated high GC content regions in CdLS-derived cells. In addition, cohesin accumulates at NIPBL-occupied sites at CpG islands potentially due to reduced cohesin translocation along chromosomes, and fewer cohesin peaks colocalize with CTCF.

To investigate whether mutated-NIPBL or -cohesin affect long-range interactions in the genome causing the transcriptional alterations, we are currently studying how mutated-NIPBL affects loop extrusion and chromatin organization around the developmental genes deregulated in CdLS.

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Searching for factors involved in the response to DNA Double Strand Breaks in late mitosis

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Our body must deal with about three thousand trillion DNA Double-Strand Breaks (DSBs) in its life, and these are among the most damaging injuries that cells face. Affected cells need to repair every DSB precisely since misrepairs can generate gross chromosome rearrangements (GCRs). These errors promote genomic instability leading to cancer, senescence or cell death. To repair DSBs, cells can carry out either non-homologous end joining (NHEJ) or homologous recombination (HR). The repair method is chosen based on cyclin-dependent kinase (CDK) levels and the availability or not of an intact homologous template. In this sense, the late stages of mitosis represent a paradoxical scenario: high CDK levels should promote HR repair while the previous segregation of sister chromatids fosters NHEJ. So far, using the model organism Saccharomyces cerevisiae, we have previously shown that, in this context, cells delay the telophase- G1 transition and seem to still favor HR over NHEJ while regrouping segregated sister chromatids (Ayra-Plasencia et al., 2019).
Now, we have undertaken a comparative proteomic analysis between late anaphase and G2/M with two different approaches to generate DSBs (phleomycin and HO endonuclease). The purpose was to find proteins that specifically change their levels during the DNA damage response in late mitosis. We will present data obtained for a selected poorly characterized protein, Msc1, that surprisingly seems to connect the DNA repair with the nuclear envelope homeostasis. Thus, the knockout MSC1 mutant presents higher sensibility to DSBs in late mitosis, and high Rad53 hyperphosphorylation, fewer Rad52 repair factories and abnormal nuclear morphologies.

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Activation of the Integrated Stress Response to overcome multidrug resistance in cancer therapy

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Resistance to therapy has been estimated to contribute to treatment failure in up to 90% of metastatic cancer patients and remains one of the fundamental challenges in cancer. Recently, we reported the surprising discovery that a set of drugs widely used in cancer therapy and with distinct targets and mechanisms of action (RAF inhibitors, etc.) are unexpectedly able to kill cancer cells showing multidrug resistance (MDR) through activation of the Integrated Stress Response (ISR). Our last findings, together with recent studies corroborating the ability of EGFR inhibitors to activate the ISR, suggest that the modulation of this pathway could be a general strategy to overcome MDR in cancer therapy. To explore this approach, we performed both chemical and genome-wide CRISPR/Cas9 knockout screens with the objective to identify new compounds and mutations that modulate the ISR. Chemical screens revealed that more than 20 FDA-approved drugs with different mechanisms of action are unexpectedly able to activate the ISR and preferentially kill tumor cells showing MDR. By interrogating different databases, we have clustered most of these ISR activators into two different groups: EGFR inhibitors (consistent with our last publication) and anthelmintic drugs (benzimidazole derivatives). In parallel, genetic screens revealed that the most potent ISR activation is achieved by knocking out genes that activate the Unfolded Protein Response (UPR). More specifically, the top hit in these screens is the chaperone HSPA5/GRP78. Since there is evidence that benzimidazole derivatives could inhibit HSPA5, in the near future we will address whether these drugs preferentially kill tumor cells showing MDR by HSPA5 inhibition while we continue to validate the rest of our screen results.

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Targeting mitochondrial translation in cancer therapy: mechanisms, genetic screens and novel drugs

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Research in recent years has shown a close relationship between increased mitochondrial translation and the resistance of cancer cells to various oncological treatments. Interestingly, mitochondrial translation can be targeted with certain antibiotics such as tigecycline, and evidences of the anticancer effects of antibiotics have been reported. However, these are isolated examples and we lack a full understanding to explain the antitumoral effects of antibiotics. Moreover, dedicated clinical trials with tigecycline showed limited efficacy, although this was thought to be in part due to bad pharmacological properties of this drug. In this context, my PhD focuses on obtaining a mechanistic understanding behind the antitumoral effects of antibiotics. Currently, I am dedicated to two independent tasks. On one end, I am conducting genetic screens to identify which mutations confer sensitivity (or resistance) to antibiotics, which should help to select the patients that should be treated with these therapies. In an independent project, I have discovered that human cells can adapt to the chronic inhibition of mitochondrial translation, and I am trying to figure out how. My progress in this project, and our future ideas, will be presented.

Replication stress recovery leads to changes in replication domain dynamics and chromatin accessibility in colorectal cancer HCT116 cells

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Replication stress is defined as the slowing or stalling of replication forks. It can cause genomic instability which is a hallmark of cancer. Cells respond to this stress by activating the replication checkpoint, leading to reversible cell cycle arrest, inhibiting late origin firing, and stabilizing the replisome. Non-transformed cells remain arrested in the S phase and are unable to recover replication after prolonged replication stress, but cancer cells can recover by firing new origins and progressing through the cell cycle.

Replication domains, which correspond to topologically associated domains, organize the replication process. Gene expression is affected by replication timing, where early replication domains belong to low-condensed chromatin regions and transcriptionally active genes, and late domains match condensed and transcriptionally repressed chromatin regions. Replication domains can switch from early to late stages or backward during development and differentiation, affecting gene expression and epigenetics.

The present work shows that a proportion of the new origins fired after severe replication stress in HCT116 cells are in new replication foci. Moreover, we found that during the recovery from severe replication stress, there was an increase in chromatin accessibility, especially in promoter regions, which can be related to replication origins. Some of these changes in accessibility were correlated with an increase in gene expression that was maintained over time. We propose a model connecting replication stress, chromatin accessibility, and gene expression variations, where the new origins fired during the recovery from severe replication stress could be a source of permanent gene expression changes that confer more heterogenicity to tumour cells.

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Investigating the impact of MeCP2 in the control of R-loops and genome integrity in cáncer

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In order to identify new factors involved in the control of R-loops metabolism, our lab recently performed a high throughput screening for factors counteracting R-loops susceptible of AID-mediated DNA breaks in U-2 OS cells. One of the top candidates identified was the Methyl CpG binding protein 2 (MeCP2). MeCP2 binds across the genome and it is enriched in regions containing GC-rich sequences and methylated CpG islands. One of the key roles of MeCP2 is the regulation of chromatin structure and transcriptional activity in part through the recruitment of different chromatin remodelers. MECP2 is highly expressed in the brain and it is essential for neural development. In fact, mutations within MECP2 are the major cause of the neurodevelopmental disorder Rett syndrome. Despite reaching the highest level of expression in the brain, MECP2 is also expressed in other tissues and its overexpression has been associated with cancer. While most of the studies regarding MeCP2 have been focused in understanding its role in the brain, very little is known about the impact of MeCP2 deregulation in the maintenance of genome integrity and carcinogenesis.

In order to validate MeCP2 as a factor implicated in R-loops regulation, we carried out siRNA mediated depletion of MECP2 in U-2 OS and HeLa cells and analyzed R-loops levels by DRIP and immunofluorescence using the S9.6 antibody. We observed increased levels of R-loops using both techniques. Moreover, MECP2 depletion in HeLa cells led to higher levels of gH2Ax foci, as well as an increase in NA breaks by comet assay that was partially suppressed upon overexpression of RNAseH1. Altogether, these results indicate that MeCP2 plays a role in maintaining genome integrity by preventing the accumulation of harmful R-loops. Our current aim is to understand the impact of MeCP2 in the maintenance of genome integrity and cell viability by controlling R-loops metabolism in different cancer cell lines as well as in RPE1 hTERT cells.

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Low-affinity CTCF binding drives transcriptional regulation whereas high-affinity binding encompasses architectural functions

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CTCF is a DNA-binding protein which plays critical roles in chromatin structure organization and transcriptional regulation; however, little is known about the functional determinants of different CTCF-binding sites (CBS). Using a conditional mouse model, we have identified one set of CBSs that are lost upon CTCF depletion (lost CBSs) and another set that persists (retained CBSs). Retained CBSs are more similar to the consensus CTCF-binding sequence and usually span tandem CTCF peaks. Lost CBSs are enriched at enhancers and promoters and associate with active chromatin marks and higher transcriptional activity. In contrast, retained CBSs are enriched at TAD and loop boundaries. Integration of ChIP-seq and RNAseq data has revealed that retained CBSs are located at the boundaries between distinct chromatin states, acting as chromatin barriers. Our results provide evidence that transient, lost CBSs are involved in transcriptional regulation, whereas retained CBSs are critical for establishing higher-order chromatin architecture.

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Exploring the potential of targeting MYC in the context of neurodegenerative diseases

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Amyotrophic Lateral Sclerosis is a deadly neurodegenerative disorder that affects motor neurons and currently lacks a cure. Previous work of our group proposed a mechanism by which the arginine-rich peptides derived from the ALS mutation in the gene C9ORF72 kill cells. In order to find therapeutic approaches for the C9- ALS pathology, we focused on seeking genetic alterations that can modulate the toxicity of these peptides. Through the isolation and analysis of clones spontaneously resistant to these peptides, we were able to identify some potential pathways which reduction was likely conferring the resistance, including mTOR and MYC pathways.

Currently, we are exploring the potential of targeting MYC as a new therapy for C9-ALS. Preliminary data suggests that reducing the Myc levels through a genetic approach is able to rescue the toxicity of arginine-rich peptides in *in vitro* and *in vivo* models. Next challenges are devoted to explore how to translate this findings into clinics, and if this concept of using anti-aging therapies against C9-ALS applies for neurodegenerative diseases in general.

Generating a human genetic atlas of Cas9-induced double strand break repair

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Our understanding of DNA repair and genome stability maintenance mechanisms has experienced a great advance in the last decade due to the possibility of easily perform genetic screens in mammalian cells thanks to the development of the CRISPR/Cas9 technology. However, an important limitation in the field has been to systematically associate the lack of particular repair factors with the precise repair outcome on the DNA molecule. To overcome this limitation, a novel CRISPR/Cas9-based screening methodology has been recently developed. This approach uses a Cas nuclease to target a double-strand break (DSB) at the backbone of a lentiviral gene sgRNA expression library previously integrated in a population of cells. In this way, the repair outcome remains spatially linked to the sequence of the sgRNA that indicates the genetic context where that repair occurred. In this work we have applied this methodology to create a genome-wide catalog of molecular repair profiles of Cas9-mediated DSBs associated to the depletion of virtually each human gene. Thus far, the analysis of the sequencing data has allowed us to identify unexpected functions of known DNA damage response (DDR) genes; non-DDR factors that affect the outcome of the repair process; genes that contribute to the editing efficiency; and mechanistic insights about the Cas9-mediated DNA editing process itself.

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The endoplasmic reticulum calcium sensor STIM1 protects cells from DNA damage

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DNA damage represents a challenge for cells, as this damage must be eliminated to preserve cell viability and the transmission of genetic information. This damage may have an endogenous origin although it may be intensified by exposure to drugs or environmental contaminants. To reduce or eliminate unscheduled chemical modifications in genomic DNA, an extensive signaling network, known as the DNA damage response (DDR) pathway ensures this repair. In this work, and by means of a proteomic analysis aimed at studying the interactome of the endoplasmic reticulum (ER) membrane protein STIM1, an ER Ca(2+) sensor, we have found that STIM1 is closely related to the protection from endogenous DNA damage, as well as in the response to interstrand crosslinks (ICLs) inducing agents. Here we show that STIM1 harbors a nuclear localization signal responsible for the spontaneous translocation to the inner nuclear membrane. Furthermore, we show that this protein translocates to the nucleus in response to mitomycin C (MMC), an ICL inducing agent, and that the pool of STIM1 bound to the chromatin also increases under conditions that trigger DNA damage. In this regard, STIM1 deficient cell lines (U2OS and HEK293) show a higher level of basal DNA damage, assessed by gamma-H2AX immunoblotting and immunofluorescence foci localization, as well as by assessing DNA breaks (comet assay). STIM1 deficiency also confers cells a greater sensitivity to aphidicolin, measured by monitoring 53BP1 foci in cyclin A2 negative cells, indicative of greater replicative stress. STIM1-KO cells were found to be more sensitive to MMC, supporting a role for STIM1 in DNA repair which is independent of the classical role of STIM1 as an activator of store operated plasma membrane Ca(2+) channels. Finally, we show that STIM1 interacts with FANCD2 and that STIM1 normalizes FANCD2 protein levels in the nucleus, a result that explains the increased sensitivity to MMC in STIM1 deficient cells.

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Exploiting cohesin STAG2 loss for clinical purposes in aggressive Ewing sarcoma

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Ewing sarcoma (EWS) is the second most frequent type of bone cancer in children. It is highly aggressive, with a survival of 30% in patients that present metastasis.
This disease is caused by a chromosomal translocation that generates an aberrant transcription factor that massively rewires the epigenome and the transcriptome, EWS-FLI1 in the 85% of the cases. In addition, loss of function mutations in cohesin STAG2 gene have been described in 20% of EWS cases, and are commonly associated with metastatic disease and poor survival outcome. Cohesin is a conserved protein complex that mediates sister chromatid cohesion and 3D genome organization and, therefore, impacts genome duplication, stability and gene expression. We hypothesize that loss of cohesin-STAG2 further rewire the malignant transcriptional program to promote metastatic Ewing sarcoma, although additional effects on genome stability cannot be discarded.
We wish to understand how STAG2 loss promotes aggressiveness in EWS, identify useful biomarkers for the early diagnosis of cases with worse prognosis and discover specific vulnerabilities in STAG2 mutant tumors that can be exploited for therapy. For that, we have defined a gene signature of 230 genes that are deregulated upon STAG2 loss both in EWS patient samples and EWS cell lines.
From these, we have selected 13 genes that are significantly associated with low survival (p-value ≤ 0.05) and show measurable changes in protein levels. We are currently evaluating their contribution to the metastatic phenotype and their potential use as biomarkers to predict aggressive cases. We are also performing drug screens to identify vulnerabilities in EWS cells lacking STAG2. From these approaches, we have identified the Lysine methyl transferase SMYD2 and the replicative CDC7 kinase as interesting candidates for therapeutic intervention in metastatic EWS. Staining of primary tumor biopsies with SMYD2 in combination with STAG2 is also being evaluated for early detection of aggressive EWS phenotype before metastasis are actually observed.

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Unrepaired Base Excision Repair Intermediates in Template DNA Strands Trigger Replication Fork Collapse and PARP Inhibitor Sensitivity

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Single-strand breaks (SSBs) are amongst the most frequent DNA lesions arising in cells and, if not repaired rapidly, can disrupt DNA replication. However, the precise mechanism/s by which unrepaired SSBs disrupt DNA replication in human cells are unclear. Here, we have exploited the exquisite sensitivity of human cells lacking either PARP1 activity or XRCC1 to the thymidine analogue 5-chloro-2′- deoxyuridine (CldU), to compare unrepaired SSBs present in nascent DNA strands with those present in template DNA strands for their impact on DNA replication.

We show that the DNA breakage, sister chromatid exchanges (SCEs), and cytotoxicity induced by CldU in the presence of PARP inhibitor or XRCC1 deletion are triggered by uracil DNA glycosylase (UNG), identifying unrepaired base excision repair (BER) intermediates in S phase as a potent source of DNA replication stress. Moreover, we show that CldU that is mis-incorporated in one cell cycle is cytotoxic only during the following cell cycle, when it is present in template DNA strands during DNA replication in the subsequent S phase.

Critically, in agreement with this, we show using DNA combing that while UNGinduced base excision repair intermediates arise both in nascent DNA strands behind replication forks and in template DNA strands ahead of replication forks, only the latter trigger replication fork collapse. Finally, we show that BRCA1- defective cells are hypersensitive to combined treatment with sub-lethal concentrations of CldU and PARP inhibitor, suggesting that CldU may have clinical utility.

Deciphering the function and dynamics of cohesins STAG1 and STAG2 during stem cell differentiation and embryo development

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Cohesin is a four-subunit complex that mediates sister chromatid cohesion and 3D genome folding. Two versions of the complex carrying STAG1 or STAG2 coexist in somatic vertebrate cells. Either one is sufficient to maintain cohesion and support cellular viability, however, STAG1 or STAG2-null mice are embryonic lethal.

Our group has previously shown that downregulation of STAG1 or STAG2 impacts differently on chromatin organization and, consequently, on gene expression. In particular, cohesin-STAG1 preferentially maintains global genome organization while cohesin-STAG2 promotes establishment of local contacts that are relevant for tissue specific transcription.

Chromatin organization is established during early embryo development and changes upon cell fate specification and differentiation, which is accompanied by drastic changes in gene expression. Our goal is to dissect the specific functions that STAG1 and STAG2 may have during these processes.

For that, we explore the dynamics of STAG1 and STAG2 during pre-implantation development, where we observe that their abundance and localization change during stages and are differential between the two variants.

Additionally, to further address STAG1 and STAG2 specificities we have chosen to study how differentiation is affected upon their removal. Differentiation of mouse embryonic stem cells has been studied in genetically deleted clones through the formation of Embryoid Bodies (EBs) by analyzing morphology, transcription and spontaneous beating. Induction of lineage-specifying genes is altered and beating of embryoid bodies is impaired, a sing of improper mesoderm differentiation.

In parallel we are setting up protocols for differentiation towards specific lineages in order to obtain homogenous populations in which we can better dissect STAG1 and STAG2 specificities using genomic technologies such as scRNA-seq, CUT&Tag and Hi-C.

Germ line mutations in STAG1 and STAG2 have been described in patients with developmental disorders. Understanding the role that each variant plays can shed light on the pathophysiology of such cohesinopathy patients.

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Estrogens modulate topoisomerase activity to control Transcription

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DNA topoisomerases are key enzymes in charge of solving topological problems that arise from essential processes of DNA metabolism, such as transcription or replication. In transcription, topoisomerases have generally been considered facilitators of the advance of RNA polymerases; however, novel specific functions in transcriptional regulation are beginning to emerge. Furthermore, in recent years, type II topoisomerases (TOP2) have been related to genome architectural proteins, suggesting a role of these enzymes in the 3D organization of complex eukaryotic genomes.

To systematically study the role of TOP2 activity in these contexts, we have developed ICEseq for the specific mapping of topoisomerase activity genome-wide.

Taking advantage of ICEseq, we have studied topoisomerase activity during the transcriptional process using estrogen-induced transcription as a model. In this work we demonstrate that TOP2 paralogs play different roles in estrogen-signaling, uncovering a new layer in hormone-mediated transcriptional control that involves a close interplay between topoisomerase activity, DNA supercoiling, and the remodeling of 3D chromatin architecture to rapidly induce gene expression.

USP7 and VCPFAF1 cooperate regulating the progression of DNA replication

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The accurate copy of the information contained in DNA is essential to assure genomic integrity. Thus, DNA replication requires fine-tuned regulatory mechanisms, involving post-translational modifications (PTMs) such as SUMOylation and Ubiquitination. In a proteomic characterisation of the human replisome, our group found an intriguing feature: whereas active DNA synthesis regions are associated with high concentrations of SUMO and low of ubiquitin, mature chromatin displays an opposite trend. We discovered that this balance is maintained by the deubiquitinase USP7 that removes ubiquitins from SUMOylated factors at replisomes. Accordingly, chemical inhibition or genetic deletion of USP7 leads to a global accumulation of ubiquitinated and SUMOylated factors on chromatin which are displaced from sites of DNA replication. We thus believe that USP7 inhibition is a good model to study replisome disassembly. Then we tried to understand how these ubiquitinated factors are evicted from replisomes. This has led us to discover that the AAA+ ATPase VCP recognises SUMOylated and ubiquitinated factors accumulated in chromatin along. The recruitment of VCP to ubiquitinylated replication factors is mediated by its adaptor FAF1, in a process that is evolutionarily conserved. Globally, our results suggest that the concentration of factors at sites of DNA replication is regulated by ubiquitin and SUMO in an equilibrium governed by USP7 and VCPFAF1.

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2022

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24/10/2022 – 26/10/2022
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MOLECULAR, CELLULAR AND ORGANISMAL DRIVERS OF AGING
09/05/2022 – 10/05/2022
Organisers: Maria A. Blasco, Alejo Efeyan, Thomas Rando

2019

HETEROGENEITY AND EVOLUTION IN CANCER
23/09/2019 – 25/09/2019
Organisers: Fátima Al-Shahrour, Arnold Levine, Solip Park, Raúl Rabadán

ESTRUCTURAL AND MOLECULAR BIOLOGY OF THE DNA DAMAGE RESPONSE
20/05/2019 – 22/05/2019
Organisers: Oscar Llorca, Rafael Fernández Leiro, Laurence H. Pearl, Titia Sixma

2018

MOLECULAR, CELLULAR AND ORGANISMAL HALLMARKS OF AGING
07/05/2018 – 09/05/2018
Organisers: Maria A. Blasco, Alejo Efeyan, Kathleen Collins, Thomas Rando

FRONTIERS IN IMMUNOMODULATION AND CANCER THERAPY
09/07/2018 – 11/07/2018
Organisers: Victoria Aranda, Nabil Djouder, Joao Monteiro, Marisol Soengas, Laurence Zitvogel

2017

PRIMARY AND SECONDARY BRAIN TUMORS
19/02/2017 – 22/02/2017
Organisers: Massimo Squatrito, Manuel Valiente, Richard Gilbertson, Michael Weller

MOLECULAR CHAPERONES IN CANCER
02/05/2017 – 04/05/2017
Organisers: Nabil Djouder, Wilhelm Krek, Paul Workman, Xiaohong Helena Yang

2016

CANCEROMATICS III – TUMOR HETEROGENEITY
13/11/2016 – 16/11/2016
Organisers: Fátima Al-Shahrour, Núria Malats, Alfonso Valencia, Chris Sander

2015

METASTASIS INITIATION:
MECHANISTIC INSIGHTS AND THERAPEUTIC OPPORTUNITIES
28/09/2015 - 30/09/2015
Organisers: David Lyden, Yibin Kang, Gemma Alderton, Victoria Aranda, Li-kuo Su, Héctor Peinado

NEW TRENDS IN ANTICANCER DRUG DEVELOPMENT
22/03/2015 - 25/03/2015
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2013

CHROMOSOME INSTABILITY AND ANEUPLOIDY IN CANCER
27/05/2013 - 29/05/2013
Organisers: Robert Benezra, Ana Losada, Marcos Malumbres, René Medema

2012

ALLOSTERIC REGULATION OF CELL SIGNALLING
17/09/2012 - 19/09/2012
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2011

RECAPTURING PLURIPOTENCY:
LINKS BETWEEN CELLULAR REPROGRAMMING AND CANCER
07/11/2011 - 09/11/2011
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CANCEROMATICS II :
MULTILEVEL INTERPRETATION OF CANCER GENOME
28/03/2011 - 30/03/2011
Organisers: Søren Brunak, Stephen Chanock, Núria Malats, Chris Sander, Alfonso Valencia

BREAST CANCER
07/02/2011 - 09/02/2011
Organisers: Joaquín Arribas, José Baselga, Miguel Ángel Piris, Lajos Pusztai and Jorge Reis-Filho

2010

CANCER PHARMACOGENETICS: PERSONALIZING MEDICINE

22/11/2010 - 24/11/2010

Organisers: Javier Benítez, William E. Evans, Miguel Martín and Magnus Ingelman-Sundberg

MOLECULAR CANCER THERAPEUTICS

08/03/2010 - 10/03/2010

Organisers: Gail Eckhardt, Roy S. Herbst and Manuel Hidalgo

2009

THE ENERGY OF CANCER

02/11/2009 - 04/11/2009

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CANCER-OM-ATICS II: MULTILEVEL INTERPRETATION OF CANCER GENOME

06/07/2009 - 08/07/2009

Organisers**:** Søren Brunak, Núria Malats, Chris Sander and Alfonso Valencia

STEM CELLS AND CANCER

23/02/2009 - 25/02/2009

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2008

SIGNALLING UPSTREAM OF mTOR
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STRUCTURE AND MECHANISMS OF ESSENTIAL COMPLEXES FOR CELL SURVIVAL
23/06/2008 - 25/06/2008
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DEVELOPMENT AND CANCER
04/02/2008 - 06/02/2008
Organisers: Konrad Basler, Ginés Morata, Eduardo Moreno and Miguel Torres

2007

LINKS BETWEEN CANCER, REPLICATION STRESS AND GENOMIC INTEGRITY
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MYC AND THE TRANSCRIPTIONAL CONTROL OF PROLIFERATION AND ONCOGENESIS
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2006

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MEDICINAL CHEMISTRY IN ONCOLOGY
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2005

CANCER AND AGING
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MAP KINASES AND CANCER
30/05/2005 - 01/06/2005
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ANIMAL TUMOUR MODELS AND FUNCTIONAL GENOMICS
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Organisers: Allan Balmain, Mariano Barbacid, Anton Berns and Tyler Jacks

2004

CADHERINS, CATENINS AND CANCER
29/11/2004 - 01/12/2004
Organisers: Amparo Cano, Hans Clevers, José Palacios and Franz Van Roy

STRUCTURAL BIOLOGY OF CANCER TARGETS
27/09/2004 - 29/09/2004
Organisers: Ernest Laue, Guillermo Montoya and Alfred Wittinghofer

2003

APOPTOSIS AND CANCER
01/12/2003 - 03/12/2003
Organisers: Gabriel Nuñez, Marisol Soengas and Scott Lowe

SMALL GTPases IN HUMAN CARCINOGENESIS
16/06/2003 - 18/06/2003
Organisers: Juan Carlos Lacal, Channing Der and Shuh Narumiya

TARGETED SEARCH FOR ANTICANCER DRUGS
17/03/2003 - 19/03/2003
Organisers: Amancio Carnero and David H. Beach

2002

MECHANISMS OF INVASION AND METASTASIS
18/11/2002 - 20/11/2002
Organisers: Maria A. Blasco and Jerry Shay

THE CELL CYCLE AND CANCER
30/09/2002 - 02/10/2002
Organisers: Marcos Malumbres, Charles Sherr and Jiri Bartek

CANCER EPIGENETICS : DNA METHYLATION AND CHROMATIN
29/05/2002 - 31/05/2002
Organisers: Manel Esteller and Stephen B. Baylin

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MEETINGS

May 22nd – 23rd , 2023
Venue: CNIO Auditorium — Madrid • Spain
Abstract submission deadline: April 20, 2023
Registration deadline: May 8, 2023

Genome organisation and stability

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MEETINGS

November 6th - 8th, 2023
Venue: CNIO Auditorium — Madrid • Spain
Abstract submission deadline: September 25, 2023
Registration deadline: October 18, 2023

Metastasis

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2024

MOLECULAR CHAPERONES IN CANCER AND PROTEIN QUALITY CONTROL

10-12 June 2024

Organisers:

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Gabriela Chiosis, Memorian Sloan Kettering Institute, US
Judith Frydman, Stanford University, US
Oscar Llorca, Spanish National Cancer Research Centre - CNIO, Spain
Paul Workman, The Institute of Cancer Research, UK

FRONTIERS IN IMMUNOMODULATION AND CANCER THERAPY: SECOND EDITION

16-18 October 2024

Organisers:

Luis Álvarez-Vallina, Spanish National Cancer Research Centre - CNIO, Spain
Maria Casanova, Spanish National Cancer Research Centre - CNIO, Spain
Nabil Djouder, Spanish National Cancer Research Centre - CNIO, Spain
David Sancho, Spanish National Centre for Cardiovascular Research – CNIC, Spain

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MEETINGS

Madrid 22nd – 23rd May 2023

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Notes

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MEETINGS

Madrid 22nd – 23rd May 2023

Genome organisation and stability

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As a non-profit organisation, we would like to thank all those who sponsored this conference. Such contribution helps us to ensure that our conferences will continue to establish the CNIO as a point of reference for the international cancer research community.

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