cnio – CaixaResearch
FRONTIERS
MEETINGS

May 28th – 30th, 2025
Venue: Spanish National Cancer Research Centre
CNIO Auditorium — Madrid • Spain

Machines acting on DNA and RNA, a molecular mechanistic perspective

Organising committee

Óscar Llorca
Spanish National Cancer Research Centre-CNIO, Spain

Rafael Fernández Leiro
Spanish National Cancer Research Centre-CNIO, Spain

Eva Nogales
University of California Berkeley, HHMI, US

Speakers

Alexey Amunts
University of Münster, Germany | EMBO Young Investigator Lecture

Gina Buchel
Yale University, US

Elena Conti
Max Planck – Biophysical Chemistry, Germany

Alessandro Costa
The Francis Crick Institute, UK

Israel S. Fernández
Biofisika Institute (CSIC-UPV/EHU), Spain

Yuan He
Johns Hopkins University, US

Siddhant Jain
Harvard Medical School, US

Leemor Joshua-Tor
Cold Spring Harbor Laboratory, US

Elizabeth Kellogg
St. Jude Children's Research Hospital, US

Meindert Lamers
Leiden University Medical Center (LUMC), Netherlands

Jun-Jie Liu
Tsinghua University, China

Nicole Hoitsma
University of Colorado Boulder, US

Lori Passmore
MRC-LMB, UK

Christian M. T. Spahn
Institute of Medical Physics and Biophysics, Germany

Alessandro Vannini
Human Technopole, Italy

Wei Yang
National Institutes of Health, NIH, US

Xiaodong Zhang
Imperial College London, UK

EXCELENCIA SEVERO OCHOA 2012 - 2024

GOBIERNO DE ESPAÑA | MINISTERIO DE CIENCIA, INNOVACIÓN Y UNIVERSIDADES | Instituto de Salud Carlos III

cnio stop cancer

"la Caixa" Foundation

Madrid 28th – 30th May 2025

Machines acting on DNA and RNA, a molecular mechanistic perspective

Spanish National Cancer Research Centre (CNIO)
Madrid, Spain

cnio stop cancer

cnio - CaixaResearch
FRONTIERS MEETINGS

Madrid 28th – 30th May 2025

Machines acting on DNA and RNA, a molecular mechanistic perspective

#CFM_MolMech
@CNIOStopCancer
@CaixaResearch

EXCELENCIA SEVERO OCHOA 2012 - 2024

GOBIERNO DE ESPAÑA | MINISTERIO DE CIENCIA, INNOVACIÓN Y UNIVERSIDADES | Instituto de Salud Carlos III

cnio stop cancer

"la Caixa" Foundation

Madrid 28th – 30th May 2025

Machines acting on DNA and RNA, a molecular mechanistic perspective

Spanish National Cancer Research Centre (CNIO)
Madrid, Spain

cnio stop cancer

cnio - CaixaResearch FRONTIERS MEETINGS

Madrid 28th – 30th May 2025

Machines acting on DNA and RNA, a molecular mechanistic perspective

Summary

07 ORGANISERS & SPEAKERS

11 PROGRAMME

SESSIONS
19 S #1 Chromatin structure and remodelling
37 S #2 DNA damage signalling and repair
55 S #3 Genome Editing
73 S #4 Macromolecular complexes in DNA / RNA processing
99 S #5a Replication and Transcription
133 S #5b Replication and Transcription
147 S #6 Translation

165 ORGANISERS & SPEAKERS' BIOGRAPHIES

187 POSTER SESSION Group 1
215 POSTER SESSION Group 2

243 Previous CNIO CaixaResearch Frontiers Meetings and CNIO Cancer Conferences

255 Notes

cnio stop cancer

Madrid 28th – 30th May 2025

Machines acting on DNA and RNA, a molecular mechanistic perspective

Spanish National Cancer Research Centre (CNIO)
Madrid, Spain

cnio stop cancer

cnio - CaixaResearch FRONTIERS MEETINGS

Madrid 28th – 30th May 2025

Machines acting on DNA and RNA, a molecular mechanistic perspective

Organisers & Speakers

cnio stop cancer

Madrid 28th – 30th May 2025

Machines acting on DNA and RNA, a molecular mechanistic perspective

Venue:

Spanish National Cancer Research Centre – CNIO Auditorium, Madrid.

Organizing committee:

Óscar Llorca
Spanish National Cancer Research Centre-CNIO, Spain

Rafael Fernández Leiro
Spanish National Cancer Research Centre-CNIO, Spain

Eva Nogales
University of California Berkeley, HHMI, US

CNIO - CaixaResearch Frontiers Meeting

Speakers

Alexey Amunts
University of Münster, Germany | EMBO Young Investigator Lecture

Gina Buchel
Yale University, US

Elena Conti
Max Planck – Biophysical Chemistry, Germany

Alessandro Costa
The Francis Crick Institute, UK

Israel S. Fernández
Biofisika Institute (CSIC-UPV/EHU), Spain

Yuan He
Johns Hopkins University, US

Siddhant Jain
Harvard Medical School, US

Leemor Joshua-Tor
Cold Spring Harbor Laboratory, US

Elizabeth Kellogg
St. Jude Children's Research Hospital, US

Meindert Lamers
Leiden University Medical Center (LUMC), Netherlands

Jun-Jie Liu
Tsinghua University, China

Nicole Hoitsma
University of Colorado Boulder, US

Lori Passmore
MRC-LMB, UK

Christian M. T. Spahn
Institute of Medical Physics and Biophysics, Germany

Alessandro Vannini
Human Technopole, Italy

Wei Yang
National Institutes of Health, NIH, US

Xiaodong Zhang
Imperial College London, UK

cnio - CaixaResearch
FRONTIERS
MEETINGS

Madrid 28th – 30th May 2025

Machines acting on DNA and RNA, a molecular mechanistic perspective

Programme

cnio stop cancer

cnio stop cancer

Wednesday May 28th, 2025

08:30 - 09:15 *Registration - main hall*

09:15 - 09:30 *Welcome address*

09:30 - 11:45 Session 1. Chromatin structure and remodelling

Chair: ***Eva Nogales***

09:30 – 10:00 *Transcriptional and extra-transcriptional roles of the RNA Polymerase III machinery in genome function and organisation*
Alessandro Vannini
Human Technopole, Italy

10:00 – 10:30 *Unraveling SMARCAD1 as a multifaceted chromatin remodeler*
Nicole Hoitsma
University of Colorado Boulder, US

10:30 - 11:00 *Poster session - coffee break – social room*

11:00 – 11:15 *short talk: Structural basis for the capture of a DNA double helix by the ATP- dependent protein clamp of a type IIA topoisomerase*
Dmitry Ghilarov
Department of Biochemistry, University of Oxford, UK

11:15 – 11:45 *Structure and Function of Mammalian SWI/SNF Chromatin Remodeling Complexes in Health and Disease*
Siddhant Jain
Harvard Medical School, US

11:45 - 15:00 Session 2. DNA damage signalling and repair

Chair: ***Lori Passmore***

11:45 – 12:15 *From RAG to NHEJ: Splicing Together Our Adaptive Immune System*
Wei Yang
National Institutes of Health-NIH, US

12:15 - 13:45 *Lunch break – Canteen*

13:45 – 14:00 *short talk: Stepwise derepression of TFIIH in global-genome nucleotide excision repair*
Natalia de Martin Garrido
The Institute of Cancer Research, UK

14:00 – 14:30 *Deciphering the Molecular Blueprint of NHEJ-Mediated DNA Repair*
Yuan He
Johns Hopkins University, US

14:30 – 15:00 *How Poxviruses target NHEJ proteins*
Óscar Llorca
Spanish National Cancer Research Centre-CNIO, Spain

15:00 - 16:00 *Poster session - coffee break – social room*

16:00 - 17:30 Session 3. Genome Editing

Chair: ***Rafael Fernández Leiro***

16:00 – 16:30 *Understanding and engineering programmable transposons for genome-editing*
Elisabeth Kellogg
St. Jude Children's Research Hospital, US

16:30 – 16:45 *short talk: Molecular and Structural Insights into AAA+-Mediated Transposase Activation*
Ernesto Arias-Palomo
Center for Biological Research (CIB) Margarita Salas, Spain

16:45 – 17:00 *short talk: Structural basis driving Tn7 target site selection*
Alba Guarné
McGill University, Canada

17:00 – 17:30 *Exploring RNA machineries for DNA manipulation*
Jun-Jie Liu
Tsinghua University, China

17:30 - 19:00 *Poster session – refreshments for all participants – social room*

Thursday May 29th, 2025

09:30 - 12:30 Session 4. Macromolecular complexes in DNA / RNA processing

Chair: ***Oscar Llorca***

09:30 – 10:00 *Molecular Machines that sense and respond to viral RNA: the RIG-I like family*
Gina Buchel
Yale University, US

10:00 – 10:15 *short talk: Helicase-mediated mechanism of SSU processome maturation and disassembly*
Sebastian Klinge
The Rockefeller University, US

10:15 – 10:45 *Molecular insights into the mRNA poly(A) tail machinery*
Lori Passmore
MRC Laboratory of Molecular Biology (LMB), UK

10:45 - 11:30 *Group picture CNIO main entrance / poster session & coffee break – social room*

11:30 – 12:00 *The RNA exosome complex at the crossroads of RNA processing and decay*
Elena Conti
Max Planck – Biophysical Chemistry, Germany

12:00 – 12:15 *short talk: Cryo-EM reveals the architecture of an active yeast m6A Methyltransferase Core Complex*
Michelangelo Lassandro
Human Technopole, Italy

12:15 – 12:30 *short talk: Molecular basis of mRNA delivery to the bacterial ribosome*
Michael Webster
John Innes Centre, UK

12:30 - 13:45 *Lunch break – Canteen*

13:45 - 17:30 Session 5a. Replication and Transcription

Chair: ***Wei Yang***

13:45 – 14:15 *Understanding the mitochondrial DNA replication machinery*
Rafael Fernández-Leiro
Spanish National Cancer Research Centre-CNIO, Spain

14:15 – 14:30 *short talk: Structural basis of transcription reduction by a promoter-proximal +1 nucleosome*
Julio Abril Garrido
Max Planck for Multidisciplinary Sciences, Germany

14:30 – 15:00 *Molecular mechanisms of ATPase-driven bacterial transcription initiation*
Xiaodong Zhang
Imperial College London, UK

15:00 – 15:15 *short talk: The bacterial helicase loader DnaI is a redox-sensing switch*
Amy Fernandez
Johns Hopkins School of Medicine, US

15:15 – 15:45 *Surprises in the modularity of large transcriptional cofactors*
Eva Nogales
University of California, Berkeley, US

15:45 - 16:15 *Poster session - coffee break – social room*

16:15 – 16:45 *Mechanism of parental nucleosome disruption and histone redistribution by the replisome*
Alessandro Costa
The Francis Crick Institute, UK

16:45 – 17:00 *short talk: Structural basis of RNA polymerase III backtracking and reactivation.*
Carlos Fernandez-Tornero
Margarita Salas Center for Biological Research, Spain

17:00 – 17:30 *To be or not to be, specific? That is the question!*
Leemor Joshua-Tor
Cold Spring Harbor Laboratory, US

17:30 - 19:00 *Poster session – refreshments for all participants – social room*

Friday, May 30th, 2025

09:30 - 10:30 Session 5b. Replication and Transcription

Chair: ***Alessandro Costa***

09:30 – 10:00 *Targeting bacterial DNA polymerases for the next generation of antibiotics*
Meindert Lamers
Leiden University Medical Center-LUMC, The Netherlands

10:00 – 10:15 *short talk: Coupling of ribosome biogenesis and translation initiation in human mitochondria*
Anna Franziska Finke
University Medical Center Goettingen, Germany

10:15 – 10:30 *short talk: Structural basis of de novo chromatin assembly during DNA replication*
Jolijn Govers
Hubrecht Institute, The Netherlands

10:30 - 12:45 Session 6. Translation

Chair: ***Alessandro Costa***

10:30 – 11:00 *Cryo-electron microscopy of actively translating ribosomes*
Christian M. T. Spahn
Institute of Medical Physics and Biophysics, Germany

11:00 – 11:15 *short talk: Mechanisms to Create Specialized Ribosomes in Bacteria*
Joaquin Ortega
McGill University, Canada

11:15 - 11:45 *Coffee break – social room*

11:45 – 12:15 *Where to start: The Dynamic Adventure of Identifying Start Condons in mRNAs*
Israel S. Fernández
Biofisika Institute (CSIC-UPV/EHU), Spain

12:15 – 12:45 *From RNA to Membrane: Mitochondrial Ribosomes at Work EMBO Young Investigator Lecture*
Alexey Amunts
University of Münster, Germany

12:45 *Poster/talk prizes*

Wrap up and closing:
Oscar Llorca
Eva Nogales and
Rafael Fernandez Leiro

Machines acting on DNA and RNA, a molecular mechanistic perspective

cnio - CaixaResearch
FRONTIERS
MEETINGS

Madrid 28th – 30th May 2025

Machines acting on DNA and RNA, a molecular mechanistic perspective

Wednesday May 28th 2025

Session #1
Chromatin structure and remodelling

Chairperson: ***Eva Nogales***

cnio stop cancer

Transcriptional and extra-transcriptional roles of the RNA Polymerase III machinery in genome function and organisation

Alessandro Vannini

Head of Structural Biology Research Centre
Human Technopole, Milan, Italy

The eukaryotic nuclear genome is transcribed by the multi-subunit enzymes RNA Polymerase (Pol) I, II and III, which catalyze DNA-dependent RNA synthesis. Pol III transcribes genes encoding short non-coding essential RNAs, such as the entire pool of transfer RNAs (tRNAs). Recent studies have shown that Pol III plays a paramount role in numerous essential processes occurring in the nucleus and in the cytoplasm, globally defined as "extra-transcriptional" functions of the Pol III transcription apparatus, since they are not strictly dependent on Pol III transcripts or on the transcriptional activity of Pol III. The lecture will focus on unpublished results unraveling unexpected mechanistic insights into extra transcriptional roles of Pol III (and SMC complexes) into chromatin organization.

Machines acting on DNA and RNA, a molecular mechanistic perspective

Notes

Machines acting on DNA and RNA, a molecular mechanistic perspective

Notes

Unraveling SMARCAD1 as a multifaceted chromatin remodeler

Nicole M. Hoitsma

Biochemistry
University of Colorado Boulder
Boulder, US

Maintaining the dynamic structure of chromatin plays a critical role in regulating cellular processes that require access to the DNA template, such as DNA damage repair, transcription, and replication. Histone chaperones and ATP-dependent chromatin remodeling factors facilitate transitions in chromatin structure by assembling and positioning nucleosomes through a variety of enzymatic activities. SMARCAD1 is a chromatin remodeler that combines the ATP-dependent ability to exchange histones with the chaperone-like activity of nucleosome deposition. We have shown that regulation of SMARCAD1 activities via its phosphorylation and its flexible N-terminal region using a battery of *in vitro* assays. Here, through a series of in-cell and *in vitro* experiments, we show that SMARCAD1 contributes to regulating active fork stability by mediating R-loop resolution. We report the binding of SMARCAD1 to R-loops *in vitro* and demonstrate its ability to facilitate their turnover in cis in the cell. Genome-wide assays reveal that cells expressing a SMARCAD1 mutant accumulate an astonishing number of R-loops compared to the wild type. As such, our data suggests SMARCAD1 as a novel sensor of R-loops at active replication forks, facilitating the resolution of transcription-replication conflicts to maintain genome stability.

Nicole M. Hoitsma[11,2], Karolin Luger[1,2], Sidrit Uruci[3,4], Nitika Taneja[3,5].
Affiliations:
1 Department of Biochemistry, University of Colorado Boulder, Boulder, CO, USA.
2 Howard Hughes Medical Institute, Chevy Chase, MD, USA.
3 Department of Molecular Genetics, Erasmus University Medical Center, Erasmus MC Cancer Institute, Rotterdam, The Netherlands.
4 Department of Human Genetics, Leiden University Medical Center, Leiden, The Netherlands.
5 Oncode Institute, The Netherlands

Machines acting on DNA and RNA, a molecular mechanistic perspective

Notes

Machines acting on DNA and RNA, a molecular mechanistic perspective

Notes

SHORT TALK

Structural basis for the capture of a DNA double helix by the ATP-dependent protein clamp of a type IIA topoisomerase

Zuzanna Pakosz-Stepien[3], Olivia Gittins[3], Jonathan G. Heddle[3] &
Dmitry Ghilarov[1,2]

1. John Innes Centre, UK
2. Department of Biochemistry, University of Oxford, UK
3. Centre for Programmable Biological Matter, Durham University, UK

Type II topoisomerases are molecular machines essential for replication, transcription and cell division in both bacteria and eukaryotes. As such, they are also key targets for antibacterial and cancer therapy. All type II topoisomerases are believed to simultaneously capture and manipulate two double-stranded segments of DNA; by cleaving the gate (G)-segment and transporting another (T) segment through the break, topoisomerase can change linking number of a DNA molecule or decatenate two DNA molecules.

The title of this abstract is an homage to a classic paper by Roca and Wang (Cell, 1992, 71:833-840) that demonstrated that upon ATP binding, type II topoisomerases form circular clamps around DNA. Despites decades of study, there is currently no direct structural evidence for how exactly an ATP-operated clamp might capture a double-stranded DNA segment. Moreover, in recent structures of a coomplete Escherichia coli DNA gyrase-DNA complex poised for strand passage from our labs the ATP-dependent clamp is completely disengaged from DNA, raising questions about the role of the clamp in enzyme catalytic cycle.

We now present a high-resolution structure of pre-strand passage (wrapped) state of a type IIA topoisomerase (DNA gyrase) from a hyperthermophilic archaeon, where the transported (T) segment DNA contiguous to the G (gate) segment DNA is captured by the ATP-dependent clamp. Contiguous nature of a DNA loop strongly suggests the determined assembly represents a native state of a functional type IIA enzyme. We describe features of the structure, compare it with one of the Gram-negative E. coli enzyme and discuss our current understanding of type IIA topoisomerase catalysis based on recent advances based on cryoEM.

Machines acting on DNA and RNA, a molecular mechanistic perspective

Notes

Machines acting on DNA and RNA, a molecular mechanistic perspective

Notes

Structure and Function of Mammalian SWI/SNF Chromatin Remodeling Complexes in Health and Disease

Siddhant Jain

Dana-Farber Cancer Institute
Harvard Medical School
Howard Hughes Medical Institute
Boston, US

ATP-dependent chromatin remodeling complexes are heterogeneous, multi-component molecular machines that govern genomic accessibility and gene expression and are frequently perturbed in human disease. This presentation highlights recent advances that have enabled the mechanistic understanding of mammalian SWI/SNF (mSWI/SNF or BAF) complex activities and interactions in developmental and disease states, opening new opportunities for targeted intervention.

Machines acting on DNA and RNA, a molecular mechanistic perspective

Notes

Machines acting on DNA and RNA, a molecular mechanistic perspective

Notes

cnio - CaixaResearch
FRONTIERS
MEETINGS

Madrid 28th – 30th May 2025

Machines acting on DNA and RNA, a molecular mechanistic perspective

Wednesday May 28th 2025

Session #2
DNA damage signalling and repair

Chairperson: ***Lori Passmore***

cnio stop cancer

From RAG to NHEJ: Splicing Together Our Adaptive Immune System

Wei Yang*

Laboratory of Molecular Biology
National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)
National Institutes of Health
Bethesda, USA

V(D)J recombination is essential for generating the adaptive immune response and unlimited number of different antibodies and antigen receptors. Encoded by multiple V, D and J gene segments, antigen receptors are assembled by programmed double-stranded DNA cleavage and imprecise re-joining. RAG1/2 recombinase initiates the process by stochastically cleaving DNA at a pair of recombination signal sequences (RSS) bordering the V, D or J gene segments. DNA double strand cleavage occurs in a single active site in two consecutive steps, hydrolysis and strand transfer, resulting in DNA hairpin on the coding end and DSB on the RSS side. Coding ends processing and joining to complete V, D, and J gene assembly and circularization of RSS end are carried out by the non-homologous end joining process (NHEJ). The DNA-dependent protein kinase (DNA-PK), consisting of the catalytic subunit (DNA-PKcs) and Ku70/80, is the key player in NJEJ by protecting broken DNA ends, promoting DNA hairpin end opening and also coordinating nucleotide removal, addition and DNA end ligation. In this seminar I will report the molecular mechanism of DNA cleavage by RAG1/2, activation of Artemis nuclease by autophosphorylated DNA-PKcs, and molecular assemblies and coordinated reactions in NHEJ.

Wei Yang*, Lan Liu, Xuemin Chen and Martin Gellert
Laboratory of Molecular Biology, NIDDK, National Institutes of Health, USA

Machines acting on DNA and RNA, a molecular mechanistic perspective

Notes

Machines acting on DNA and RNA, a molecular mechanistic perspective

Notes

SHORT TALK

Stepwise derepression of TFIIH in global-genome nucleotide excision repair

Natàlia de Martín Garrido[1], Callum Haste[1], Junjie Feng[1], Nora B. Cronin[2], Basil J. Greber[1]

1. Division of Structural Biology, The Institute of Cancer Research, 237 Fulham Road, London SW3 6JB, UK
2. London Consortium for High Resolution Cryo-EM, The Francis Crick Institute, London NW1 1AT, UK

Nucleotide excision repair (NER) is a crucial DNA repair pathway that is orchestrated by transcription factor IIH (TFIIH) in eukaryotic cells. TFIIH is a multifunctional complex that contains two DNA helicase/DNA translocase subunits and an associated kinase module, different subsets of which act in NER, transcription initiation, and cell cycle control. To ensure fidelity despite multifunctionality, the DNA helicase activity in TFIIH is auto-inhibited in its free form or when the factor engages in transcription. While the release of the kinase module has been identified as a key step in TFIIH activation, the molecular mechanisms that control this step and concomitant structural changes in TFIIH are poorly understood. Several studies that have characterised early steps of the NER process after TFIIH recruitment omit the CAK subcomplex and have used crosslinking agents during sample preparation for cryo-EM, therefore preventing the visualisation of dynamic transitions. To facilitate the structural characterisation of dynamic intermediates, we used streptavidin affinity grids and a lesion-containing biotinylated DNA to tether molecular complexes assembling around this DNA lesion on cryo-EM grids.

Using this strategy, we have determined high-resolution structures of several intermediates of the early stages of global genome NER that were previously unknown. Our structures reveal how holo-TFIIH, in the presence of CAK, is initially recruited to DNA lesions by the XPC complex in an auto-inhibited state, and de-repressed in a stepwise manner by binding of the protein XPA and by dynamic conformational changes within TFIIH itself. Furthermore, our results show that the presence of CAK and XPA on TFIIH is not mutually exclusive, supporting previous data suggesting the requirement for ATP in the release of the inhibitory CAK module. Altogether, these findings contribute to a deeper mechanistic understanding of NER, a critical DNA repair pathway that ensures genome integrity.

Machines acting on DNA and RNA, a molecular mechanistic perspective

Notes

Machines acting on DNA and RNA, a molecular mechanistic perspective

Notes

Deciphering the Molecular Blueprint of NHEJ-Mediated DNA Repair

Yuan He
Thomas C. Jenkins Department of Biophysics
Johns Hopkins University
Baltimore, US

DNA double-strand breaks (DSBs) pose an existential threat to genomic integrity; their erroneous repair drives tumorigenesis, whereas their programmed resolution underpins V(D)J recombination. In metazoans, most DSBs are repaired by non-homologous end joining (NHEJ), yet the size and plasticity of the NHEJ apparatus have obscured a molecular description of its reaction pathway. We integrate single-particle cryo-electron microscopy (cryo-EM), single-molecule Förster resonance energy transfer (smFRET) and biochemical reconstitution to delineate the sequential synapsis of human DSB ends. Cryo-EM reconstructions reveal an initial long-range complex in which two Ku70/80–DNA-PKcs modules capture DNA ends ~115 Å apart and are bridged by an XRCC4-XLF–DNA ligase IV (LigIV) scaffold. Face-to-face juxtaposition of the DNA-PKcs kinase domains enables trans-autophosphorylation, licensing DNA-PKcs dissociation and collapse into a short-range complex that co-axially aligns the ends. A single LigIV catalytic domain engages a nick between Ku rings, implying alternating action of the two LigIV subunits to seal complementary strands. Time-resolved smFRET combined with cryoEM visualizes rapid, reversible transitions between the long- and short-range states and captures nucleases, polymerases and kinase modules docking within the Ku-flanked channel without disrupting synapsis. Our data support a model in which NHEJ operates as a malleable reaction chamber that sequentially trims, fills and ligates DSBs while safeguarding end alignment, providing a structural framework for therapeutic modulation of genome repair.

Machines acting on DNA and RNA, a molecular mechanistic perspective

Notes

Machines acting on DNA and RNA, a molecular mechanistic perspective

Notes

How Poxviruses target NHEJ proteins

Óscar Llorca
Structural Biology Programme Director
Macromolecular Complexes in DNA Damage Response Group
Spanish National Cancer Research Centre (CNIO)
Madrid, Spain

The Ku70/Ku80 heterodimer is a central component of the non-homologous end-joining (NHEJ) pathway, responsible for detecting and repairing DNA double-strand breaks in the nucleus. Interestingly, beyond its nuclear role, Ku70/Ku80 also functions in the cytoplasm as a DNA sensor that detects viral DNA and activates innate immune responses. To counteract this antiviral function, many pathogens, including poxviruses, have evolved strategies to evade or inhibit Ku70/Ku80 sensing. The vaccinia virus (VACV), a prototypical member of the Poxviridae family that replicates its double-stranded DNA genome in the cytoplasm of host cells, expresses two viral proteins that specifically interact with Ku70/Ku80 to subvert its immune surveillance role and promote viral replication and virulence.

Using cryo-electron microscopy (cryo-EM), we have determined the structure of Ku70/Ku80 in complex with these VACV-encoded proteins, uncovering the molecular basis by which VACV manipulates this DNA repair factor. Our structural and functional analyses reveal how these viral proteins interfere with Ku70/Ku80-mediated immune activation. Furthermore, we identify conserved mechanisms among orthopoxviruses, including variola virus, the agent causing smallpox, and monkeypox virus, suggesting a broadly conserved strategy for immune evasion across the poxvirus family.

Notes

Machines acting on DNA and RNA, a molecular mechanistic perspective

Notes

cnïo - CaixaResearch
FRONTIERS
MEETINGS

Madrid 28th – 30th May 2025

Machines acting on DNA and RNA, a molecular mechanistic perspective

Wednesday May 28th 2025

Session #3
Genome Editing

Chairperson: ***Rafael Fernández Leiro***

cnïo stop cancer

Understanding and Engineering transposons for next-generation genome-editing

Dr. Elizabeth (Liz) Kellogg

Department of Structural Biology
Jude Children's Research Hospital
Memphis, US

CRISPR-associated transposition systems allow guide RNA-directed integration of a single DNA insertion in one orientation at a fixed distance from a programmable target sequence. These transposition systems are highly complex, with multiple components interacting to carry out a highly coordinated sequence of events to select target-sites and subsequently integrate cargo DNA. The Kellogg lab seeks to understand the underlying mechanisms of programmable DNA-insertion by using high-resolution single-particle Cryo-EM to characterize the structure of these and related transposition systems. The Kellogg lab further seeks to harness their mechanistic insights for engineering novel transposon systems for genome-editing applications.

Notes

Machines acting on DNA and RNA, a molecular mechanistic perspective

Notes

SHORT TALK

Molecular and Structural Insights into AAA+-Mediated Transposase Activation.

Álvaro de la Gándara, Mercedes Spínola-Amilibia, Lidia Araújo-Bazán, Rafael Núñez- Ramírez, James M. Berger, **Ernesto Arias-Palomo**
Centro de Investigaciones Biológicas Margarita Salas, CSIC, Madrid, Spain.
Department of Biophysics and Biophysical Chemistry, Johns Hopkins University School of Medicine, Baltimore, MD, USA.

Transposases drive chromosomal rearrangements and the dissemination of drugresistance genes and toxins. Although some transposases act alone, numerous classes rely on dedicated AAA+ ATPase subunits that regulate site selectivity and catalytic function through poorly understood mechanisms. Here, we have characterized a widespread and streamlined family of transposons, IS21, which contains two genes: the transposase, IstA, and an essential AAA+ regulator, IstB.

We have used solution and cryo-EM studies to determine that isolated IstA and IstB self-assemble with their cognate nucleic acids into inactive complexes to prevent futile ATPase cycles and deleterious chromosomal breaks. Moreover, a 3.6 Å-resolution structure of a ~1 MDa transpososome complex shows how the ATPase-regulator uses nucleotide-controlled assembly and DNA deformation to enable site selectivity, transposase recruitment/activation, and integration.

Importantly, specific transposase-regulator interactions stimulate ATPase activity and trigger a large conformational change on the transposase that positions the catalytic site with the correct configuration to perform DNA strand transfer. These studies help explain how AAA+ ATPase regulators – which are used by classic transposition systems and CRISPR-associated elements – can remodel their substrate DNA and cognate transposases to promote function.

Machines acting on DNA and RNA, a molecular mechanistic perspective

Notes

Machines acting on DNA and RNA, a molecular mechanistic perspective

Notes

SHORT TALK

Structural basis driving Tn7 target site selection.

Yao Shen[1,3], Shreya Krishnan[1], Michael T. Petassi[2], Joseph E. Peters[2], **Alba Guarné[1,*]**

1. Department of Biochemistry and Centre de Recherche and Biologie Structurale, McGill University, Montreal (QC), Canada.
2. Department of Microbiology, Cornell University, Ithaca (NY), USA.
3. Present address: Division of Structural Biology, Centre for Human Genetics, University of Oxford, Oxford, UK.

Transposons are DNA fragments that can move autonomously within genomes. While many lack target specificity, others are characterized by their precise insertions and could be used to move DNA cargo to specific genome locations. Tn7 transposons are characterized by their sophisticated target-site selection mechanisms, precise insertions, and, in some cases, programmability. The latter makes them attractive systems for developing genetic tools and potential genetic engineering applications. Tn7 targeting to specific chromosomal sites relies on the coordinated actions of specific target-site selection proteins and the AAA+ adaptor TnsC to recruit and activate the transposase (TnsAB) at specific sites. Prototypical Tn7 encodes two target-site selection proteins that direct insertions to either a single chromosomal site (TnsD) or to replicating conjugal plasmids (TnsE).

Combining cryo-electron microscopy and X-ray crystallography with other biophysical and biochemical techniques, we found that the stepwise assembly of Tn7 target site selection complexes ensures that Tn7 only targets sites that do not harbour a pre-existing Tn7 element (Shen et al. (2024) Molecular Cell; Krishnan et al. (2025)), and insertions occur at a strict distance from the target site (Shen et al. (2022) NSMB). These findings provide a critical mechanistic and explain why Tn7 is more specific than other transposons, a feature that is essential to developing transposons into genetic tools.

Notes

Machines acting on DNA and RNA, a molecular mechanistic perspective

Notes

Exploring RNA machineries for DNA manipulation

Jun-Jie Gogo Liu
Associate Professor
School of Life Sciences
Tsinghua University
Beijing, China

RNA, as a critical biological macromolecule, plays an indispensable role in diverse biological processes by regulating or guiding protein functions and even directly catalyzing biochemical reactions. In practical applications, RNA elements are typically more programmable and manipulable than proteins, providing novel dimensions and possibilities for functional carriers in biotechnology. In my presentation, I will discuss our focus on RNA elements to identify new bio-molecular machineries, such as retrotransposons and ribozymes, and utilize biochemical and structural approaches to elucidate their mechanisms of action for potential applications in biomolecular manipulation, including gene editing.

Machines acting on DNA and RNA, a molecular mechanistic perspective

Notes

Machines acting on DNA and RNA, a molecular mechanistic perspective

Notes

cnio - CaixaResearch FRONTIERS MEETINGS

Madrid 28th – 30th May 2025

Machines acting on DNA and RNA, a molecular mechanistic perspective

Thursday May 29th 2025

Session #4
Macromolecular complexes in DNA / RNA processing

Chairperson: ***Oscar Llorca***

Machines acting on DNA and RNA, a molecular mechanistic perspective

Molecular Machines that sense and respond to viral RNA: the RIG-I like family

Gina Buchel
Department of Molecular, Cellular, and Developmental Biology
Yale University
New Haven, US

A first line of defense against RNA viruses is the innate immune receptor MDA5, which is activated upon binding viral double-stranded RNA (dsRNA), triggering a signaling cascade to eliminate the pathogen from the host. While critical for antiviral defense, dysregulation of MDA5 has been implicated in various autoimmune and inflammatory diseases. MDA5 is known to work cooperatively with a regulatory protein known as LGP2, and it has been hypothesized that these proteins function by forming a heteromeric complex on dsRNA to enhance MDA5 activity. However, the specific interactions that take place within this complex remain unclear. In this study, we elucidate the mechanism for LGP2 coactivation of MDA5. We use a combination of *in vitro* biochemical methods and *in cellulo* approaches to investigate the molecular basis of MDA5-LGP2-dsRNA complex assembly. Altogether, our work provides valuable foundational knowledge pertaining to the onset of autoimmune and inflammatory diseases, thereby offering potential pathways for developing MDA5-targeted therapeutic antagonists. Additionally, by elucidating the molecular basis of MDA5 activation and its regulation, the knowledge can be utilized for the development of efficacious cancer therapeutics, as immunomodulation has promising potential in promoting anti-tumor immunity in numerous cancer models.

Machines acting on DNA and RNA, a molecular mechanistic perspective

Notes

Machines acting on DNA and RNA, a molecular mechanistic perspective

Notes

SHORT TALK

Helicase-mediated mechanism of SSU processome maturation and disassembly

Olga Buzovetsky & **Sebastian Klinge**
The Rockefeller University

Eukaryotic ribosomal small subunit (SSU) assembly requires the SSU processome, a nucleolar precursor containing the RNA chaperone U3 snoRNA. The underlying molecular mechanisms of SSU processome maturation, remodeling, disassembly, RNA quality control, and the transitions between states remain elusive due to a paucity of intermediates. Here we report 16 native SSU processome structures alongside genetic data, revealing how two helicases, the Mtr4-exosome and Dhr1, are controlled for accurate and unidirectional ribosome biogenesis. Our data show how irreversible pre-ribosomal RNA degradation by the redundantly tethered RNA exosome couples the transformation of the SSU processome into a pre-40S particle during which Utp14 can probe evolving surfaces, ultimately positioning and activating Dhr1 to unwind the U3 snoRNA and initiate nucleolar pre-40S release.
This study highlights a paradigm for large dynamic RNA-protein complexes where irreversible RNA degradation drives compositional changes and communicates these changes to govern enzyme activity while maintaining overall quality control.

Machines acting on DNA and RNA, a molecular mechanistic perspective

Notes

Machines acting on DNA and RNA, a molecular mechanistic perspective

Notes

Molecular insights into the mRNA poly(A) tail machinery

Lori Passmore
MRC Laboratory of Molecular Biology
Cambridge, UK

Cytoplasmic shortening of mRNA poly(A) tails represses eukaryotic gene expression by inhibiting efficient translation and committing an mRNA to decay. This occurs in gene- and context-dependent manners to allow transcript-specific control of poly(A) tail lengths. Understanding how specific mRNAs are targeted for deadenylation has been a central question in the field of gene expression for many years. Specificity is achieved through RNA adaptors – RNA-binding proteins that recruit the CCR4-NOT deadenylase machinery to certain substrate mRNAs in a regulated manner to target specific transcripts for deadenylation. Examples of RNA adapters are Pumilio/Puf proteins, TTP/tristetraprolin and the microRNA-induced silencing complex (miRISC). On the other hand, the PAN2–PAN3 deadenylation complex is generally thought to act non-discriminately on long poly(A) tails. Still, the molecular mechanisms of specificity remain unclear in many cases. We use structural biology and biochemical reconstitution to approach this problem. We find that RNA adapters are much more widespread than previously thought. Second, we find that RNA adapters interact with CCR4-NOT via multivalent interactions with their intrinsically disordered regions (IDRs). Finally, these interactions can be regulated to mediated tuning of deadenylation rate. Together, this contributed to the intricate regulation of gene expression.

Machines acting on DNA and RNA, a molecular mechanistic perspective

Notes

Machines acting on DNA and RNA, a molecular mechanistic perspective

Notes

The RNA exosome complex at the crossroads of RNA processing and decay

Elena Conti
Max Planck Institute of Biochemistry
Structural Cell Biology Department
Munich, Germany

The RNA exosome is a conserved multiprotein complex essential for 3'-5' RNA degradation in eukaryotic cells. In the cytoplasm, the exosome participates in mRNA quality-control pathways linked to translating ribosomes, while in the nucleus it performs a broad range of functions, from fully degrading cryptic RNAs generated by faulty transcription to processing precursor transcripts. An extended network of obligate cofactors and transient RNA helicase complexes has evolved to handle the large variety of RNA substrates in each subcellular compartment. This network organizes in layers around the exosome core and regulates the irreversible degradative action in synergy with the features of the substrates. In the cytoplasm, exosome core associates with the obligate cofactor HBS1L3/SKI7, and transiently recruits the SKI238 helicase complex. Using biochemical and structural approaches, we uncovered how the human exosome holo-complex can extract and degrade a ribosome-bound mRNA. Interestingly, the SKI238 complex also appears to establish a recognition platform in the context of collided disomes. Exosome and ribosome thus work together as a single structural and functional unit in co-translational mRNA decay, coordinating their activities in a transient supercomplex.

Machines acting on DNA and RNA, a molecular mechanistic perspective

Notes

Machines acting on DNA and RNA, a molecular mechanistic perspective

Notes

SHORT TALK

Cryo-EM reveals the architecture of an active yeast m6A Methyltransferase Core Complex

Michelangelo Lassandro[1], Antonio Sponga[1], Giulia Salzano[1], Waleed S Albihlal[2], Andrea Graziadei[1], Sebastiano Pasqualato[1], Paolo Swuec[1], Folkert J van Werven[2], Ana Casañal[1]

(1) Human Technopole, Milan, Italy
(2) The Francis Crick Institute, London, United Kingdom

RNA modifications are critical post-transcriptional regulators influencing diverse biological processes. In eukaryotes, m6A methylation is mediated by the multiprotein Methyltransferase Complex (MTC). Despite structural insights into individual subcomplexes, the full composition and architecture of an active m6A methyltransferase complex remain unclear.

Here, we present the architecture of the active yeast m6A Methyltransferase Core Complex (yMTcc). This assembly is composed of five conserved subunits, necessary and sufficient for mRNA methylation in Saccharomyces Cerevisiae: the catalytic subunit Ime4 with Kar4, Mum2, Vir1, and Slz1 (METTL3, METTL14, WTAP, VIRMA, and ZC3H13, in human). Cryo-EM analysis reveals a 2.6 Å reconstruction of the active yMTcc. Our model, supported by Crosslinking-MS and SEC-MALLS, defines the stoichiometry of the machinery: Vir1 and Mum2 assemble in multiple copies alongside Ime4, Kar4 and Slz1. Notably, the enzymatic subunit Ime4 acts as a scaffold, organizing the subunits into a compact assembly. We demonstrate that the interactions between Ime4 and Mum2 are crucial for maintaining the functions of the yMTcc *in vivo*. Interestingly, the same interaction platform appears to be conserved between the human orthologues METTL3 and WTAP, as suggested by AlphaFold3 prediction.

This detailed structural study of yMTcc, with its unexpected stoichiometry, advances our understanding of the m6A machinery evolution and function across eukaryotes and its implications in gene regulation.

Machines acting on DNA and RNA, a molecular mechanistic perspective

Notes

Machines acting on DNA and RNA, a molecular mechanistic perspective

Notes

SHORT TALK

Molecular basis of mRNA delivery to the bacterial ribosome

Michael W. Webster[1,2], Adrien Chauvier[3], Huma Rahil[2], Andrea Graziadei[4], Kristine Charles[4], Nataliya Miropolskaya[2], Maria Takacs[2], Charlotte Saint-André[2], Juri Rappsilber[4,5], Nils G. Walter[3], Albert Weixlbaumer[2]

1. Department of Biochemistry and Metabolism, John Innes Centre, Norwich Research Park, Norwich, UK.
2. Department of Integrated Structural Biology, Institut de Génétique et de Biologie Moleculaire et Cellulaire (IGBMC), Illkirch Cedex, France.
3. Single Molecule Analysis Group, Department of Chemistry and Center for RNA Biomedicine, University of Michigan, Ann Arbor, MI, USA.
4. Technische Universität Berlin, Chair of Bioanalytics, Berlin, Germany.
5. Wellcome Centre for Cell Biology, University of Edinburgh, Max Born Crescent, Edinburgh, UK.

Protein synthesis begins with formation of a ribosome-mRNA complex. In bacteria, the 30S ribosomal subunit is recruited to many mRNAs through both base pairing with the Shine Dalgarno sequence and RNA-binding by ribosomal protein bS1.

Translation can initiate on mRNAs that are being transcribed, and binding of the pioneering 30S subunit can also be promoted by RNA polymerase. Despite their significance to the translation initiation pathway, our mechanistic understanding of the complementary roles of the Shine Dalgarno sequence, bS1 and RNA polymerase remain limited.

Here, I will present our model of how ribosomes are recruitment to mRNAs and transcription complexes in bacteria. Using cryo-EM, we determined structural models that reveal how bS1 delivers mRNAs to the ribosome for Shine Dalgarno duplex formation. The structural data also suggest mRNA delivery is coordinated with initial 30S subunit activation through interactions of bS1 with RNA. We characterised the kinetics of 30S binding to mRNAs and transcription complexes using single-molecule fluorescence co-localization experiments, which showed that bS1 also mediates the stimulation of translation initiation by RNA polymerase.

Finally, in-cell crosslinking mass spectrometry showed that the interactions between 30S and RNA polymerase we identified *in vitro* also occur within E. coli cells.

These findings provide a mechanistic framework for the initiation of bacterial translation and establishment of transcription-translation coupling [1].

[1] Webster et al. (2024) Molecular basis of mRNA delivery to the bacterial ribosome. Science 386:eado8476. PMID: 39607923.

Machines acting on DNA and RNA, a molecular mechanistic perspective

Notes

Machines acting on DNA and RNA, a molecular mechanistic perspective

Notes

cnio - CaixaResearch
FRONTIERS
MEETINGS

Madrid 28th – 30th May 2025

Machines acting on DNA and RNA, a molecular mechanistic perspective

Thursday May 29th 2025

Session #5a
Replication and Transcription

Chairperson: ***Wei Yang***

Machines acting on DNA and RNA, a molecular mechanistic perspective

Understanding the mitochondrial DNA replication machinery

Rafael Fernández-Leiro

Genome Integrity and Structural Biology Group
Structural Biology Programme
Spanish National Cancer Research Centre (CNIO)
Madrid, Spain

Recent evidence directly links mitochondrial metabolism to neurological and neurodegenerative disorders. Mutations in nuclear genes essential for mitochondrial DNA (mtDNA) maintenance lead to severe neurological conditions. Moreover, mtDNA structural defects have also been linked to neurodegenerative disorders and cancer progression. The replication of mtDNA by the mitochondrial DNA replisome is the primary process that ensures mtDNA integrity and is vital for mitochondrial function; however, its mechanism remains largely unexplored. Understanding the mtDNA replication process is crucial for deciphering the pathogenesis of related disorders and for developing treatments. In this talk, I will summarise our efforts to study this system, focusing on recent discoveries regarding the DNA polymerase gamma.

Machines acting on DNA and RNA, a molecular mechanistic perspective

Notes

Machines acting on DNA and RNA, a molecular mechanistic perspective

Notes

SHORT TALK

Structural basis of transcription reduction by a promoter-proximal +1 nucleosome

Julio Abril Garrido, Christian Dienemann, Frauke Grabbe, Taras Velychko, Michael Lidschreiber, Haibo Wang, Patrick Cramer
Max Planck Institute for Multidisciplinary Sciences, Department of Molecular Biology (Göttingen, Germany)
Zhejiang University School of Medicine (Hangzhou, China)

At active human genes, the +1 nucleosome is located ~20-60 bp downstream of the transcription start site (TSS), near the promoter region where the RNA polymerase II (Pol II) pre-initiation complex (PIC) assembles. However, at inactive genes, the +1 nucleosome is found further upstream, at a promoter-proximal location. Here we provide evidence that a promoter-proximal +1 nucleosome can reduce RNA synthesis *in vivo* and *in vitro* and we analyze the structural basis for this effect. We find that the PIC assembles normally when the edge of the +1 nucleosome is located 18 base pairs (bp) downstream of the TSS. However, when the nucleosome edge is located further upstream, only 10 bp downstream of the TSS, the PIC adopts an inhibited state. The transcription factor IIH (TFIIH) shows a closed conformation and its subunit XPB contacts DNA with only one of its two ATPase lobes, inconsistent with DNA opening. These results provide a mechanism for nucleosome-dependent regulation of transcription initiation

Notes

Machines acting on DNA and RNA, a molecular mechanistic perspective

Notes

Molecular mechanisms of ATPase-driven bacterial transcription initiation

Xiaodong Zhang

Professor of Macromolecular Structure and Function
Imperial College London, https://profiles.imperial.ac.uk/xiaodong.zhang &
The Francis Crick Institute https://www.crick.ac.uk/research/labs/xiaodong-zhang

Transcription initiation is a highly dynamic process, involving double-stranded DNA being opened up and single template strand being delivered to the active site. We use a specialised form of bacterial RNA polymerase, which requires activators belonging to the large ATPases associated with diverse cellular activities (AAA+) family, to study this dynamic process. We have captured a series of structures representing different functional intermediates during initiation, unravelling the structural basis and molecular mechanisms for this fundamental process. Our studies reveal a remarkable macromolecular machine that undergoes large dynamic conformational transformations to manipulate DNA, leading to DNA opening, transcription bubble formation and eventually productive transcription. I will present our published and unpublished work that addresses the remarkable process that is enabled by the AAA+ ATPase machine.

Machines acting on DNA and RNA, a molecular mechanistic perspective

Notes

Machines acting on DNA and RNA, a molecular mechanistic perspective

Notes

SHORT TALK

The bacterial helicase loader DnaI is a redox-sensing switch

Amy Fernandez, Dan Richman, James Berger
Department of Biophysics and Biophysical Chemistry, Johns Hopkins School of Medicine, US

Bacterial DNA replication initiation is triggered by the loading of hexameric helicases onto DNA by dedicated AAA+ ATPases. While this step is essential, how it is regulated under times of cellular stress is unknown. Here, we show that a bacterial helicase loader, DnaI, binds an oxygen sensitive iron-sulfur cluster that controls its ability to load the helicase onto DNA and function as an ATPase. Cryo- EM structures of oxidized and reduced helicase-loader complexes show that cluster oxidation leads to conformational changes in a hinge region of the loader, reorienting its ATPase domains and affecting its ability crack open the helicase. Comparative studies of diverse bacterial helicase loaders show that FeS cluster binding may be widespread, suggesting that this is a conserved mechanism of managing DNA-damage causing oxidative stress during replication.

Notes

Machines acting on DNA and RNA, a molecular mechanistic perspective

Notes

Surprises in the modularity of large transcriptional cofactors

Eva Nogales
University of California, Berkeley
Dept. of Molecular & Cell Biology
Berkeley, CA US

Transcriptional coactivator complexes activate genes by modifying chromatin, either through post-translational modification of histones or ATP-dependent remodeling of nucleosomes. These coactivators are typically large protein assemblies composed of well-defined modules, including histone state readers, transcription factor binding domains, enzymatic activities, and structural scaffolds that loosely and flexibly hold the complex together. These functional modules are often highly conserved across eukaryotes, and some are shared among multiple coactivators within a single cell. Our lab has used cryo-electron microscopy (cryo-EM) to structurally characterize several of these coactivators and has observed surprising differences in module organization between yeast and human complexes. These findings highlight the versatility of coactivator assembly and contrast with the high conservation seen in the general RNA polymerase II (Pol II) transcriptional machinery.

Machines acting on DNA and RNA, a molecular mechanistic perspective

Notes

Machines acting on DNA and RNA, a molecular mechanistic perspective

Notes

Mechanism of parental nucleosome disruption and histone redistribution by the replisome

Alessandro Costa
Macromolecular Machines Laboratory
The Francis Crick Institute
London, UK

Eukaryotic chromosomes are packaged into chromatin, a nucleoprotein ultrastructure that protects and regulates the genome. The building blocks of chromatin are nucleosomes, formed of DNA wrapped around a core of eight histones, whose post-translational modifications play activating or repressive roles that modulate gene expression. This epigenetic programme is inherited during chromosome replication, ensuring the maintenance of cell identity and correct organismal development. To achieve epigenetic inheritance, histones must be extracted from parental nucleosomes and redeposited onto duplicated DNA at the advancing replication fork. How the replication machinery achieves this task is largely unknown at the molecular level. To start to address this, we took a biochemical reconstitution approach to investigate the kinetics of nucleosome disruption and parental histone retention at the replication fork. We used cryo-EM to visualise the encounter of a nucleosome by the replication machinery. We validated our structure-derived mechanism using chromatin replication assays reconstituted with purified yeast proteins and genomic analysis in yeast and mouse embryonic stem cells.

Machines acting on DNA and RNA, a molecular mechanistic perspective

Notes

Machines acting on DNA and RNA, a molecular mechanistic perspective

Notes

SHORT TALK

Structural basis of RNA polymerase III backtracking and reactivation

Sonia Huecas, Adrián Plaza-Pegueroles, **Carlos Fernández-Tornero**
Centro de Investigaciones Biológicas Margarita Salas, CSIC, 28040 Madrid, Spain

RNA polymerases pause during transcription due to various signals, such as errors in the transcript, specific downstream DNA sequences or DNA-bound proteins.
Pausing induces reverse translocation of RNA polymerase, which inactivates the enzyme and requires RNA cleavage for reactivation. Eukaryotic cells use RNA polymerase III (Pol III) to transcribe tRNAs, the spliceosomal U6 RNA and other short and abundant RNAs that perform essential cell functions. Pol III is composed of 17 subunits with a total mass of 700 kDa. In contrast to other RNA polymerases, Pol III rescue from a backtracked state resides in an internal module of the enzyme located in the C-terminal domain of its C11 subunit. We used cryo- EM combined with biochemical analysis to characterize different stages of RNA cleavage by Pol III. Our results show that paused Pol III interacts more loosely with the hybrid formed by the template DNA strand and the newly-synthetized RNA.
Moreover, the C-terminal domain of subunit C11 and the Pol III catalytic center coordinate to cleave backtracked RNA nucleotides. The high resolution of our structures allows to obtain mechanistic insights into Pol III backtracking and reactivation.

Notes

Machines acting on DNA and RNA, a molecular mechanistic perspective

Notes

To be or not to be, specific? That is the question!

Leemor Joshua-Tor
Howard Hughes Medical Institute
Cold Spring Harbor Laboratory
New York, US

DNA replication in eukaryotes is initiated from sites called 'origins of replication' and starts with the assembly of a pre-initiation complex (pre-IC). The first component of the pre-IC is a complex called the Origin Recognition Complex (ORC), composed of six proteins, ORC1-6, followed by the recruitment of CDC6. In the yeast, Saccharomyces cerevisiae, binding to the origin is sequence specific, while in metazoans, such as humans and flies, it appears not to be the case. I will discuss origin binding by human ORC and another yeast, which appears to have features intermediate between S. cerevisiae and humans.

Jack Bauer[1,2,3], Om Prakash Chouhan[1,3], Narges Zali[1,4], Osama El Damerash[1], Bruce Stillman[1] and Leemor Joshua-Tor[1,3]
1. Cold Spring Harbor Laboratory, 1 Bungtown Road, Cold Spring Harbor, NY 11724
2. Graduate Program in Molecular and Cellular Pharmacology, Stony Brook University, Stony Brook, NY 11794
3. Howard Hughes Medical Institute
4. Graduate Program in Genetics, Stony Brook University, Stony Brook, NY 11794

Machines acting on DNA and RNA, a molecular mechanistic perspective

Notes

Machines acting on DNA and RNA, a molecular mechanistic perspective

Notes

Madrid 28th – 30th May 2025

Machines acting on DNA and RNA, a molecular mechanistic perspective

Friday May 30th 2025

Session #5b
Replication and Transcription

Chairperson: ***Alessandro Costa***

Machines acting on DNA and RNA, a molecular mechanistic perspective

Targetting bacterial DNA polymerases for the next generation of antibiotics

Meindert Lamers

Co-director NeCEN & Associate professor Leiden University Medical Center (LUMC)
Institute of Biology Leiden
The Netherlands

The steep rise in antibiotic resistance is a major threat to global health, with estimates of 10 million deaths annually due to drug-resistant bacteria by 2050. Therefore, there is an urgent need for new antibiotics that inhibit novel targets. Replicative DNA polymerases are attractive targets due to their essential role but have not been used as targets for antibiotics. In contrast, viral replicases such as the reverse transcriptases and RNA dependent RNA polymerases have been targeted extensively for antiviral drugs. In this talk, I will present our recent work on two inhibitors of bacterial replicative DNA polymerases: nargenicin that inhibits the DnaE1 polymerase from the major pathogen *Mycobacterium tuberculosis* and ibezapolstat that inhibits the PolC polymerase from Gram-positive pathogens such as Staphylococcus aureus, *Streptococcus pyogenes* and Clostridioides difficile. Cryo-EM structures of nargenicin-bound DnaE1 and ibezapolstat-bound PolC show novel, but distinct modes of inhibition. Nargenicin occupies both the template base and incoming base and thus acts as a 'base pair analogue'. In contrast, ibezapolstat acts like a classical nucleotide analogue, yet inserts an aromatic moiety into an induced pocket of the polymerase active site. This induced pocket is not found in DnaE polymerases from Gram-negative bacteria or eukaryotic DNA polymerases Pol delta or Pol epsilon, explaining ibezapolstat's selective inhibition of Gram-positive PolC polymerases. These structures provide essential information for the further development of urgently needed novel antibiotics in the fight against drug-resistance bacteria.

Machines acting on DNA and RNA, a molecular mechanistic perspective

Notes

Machines acting on DNA and RNA, a molecular mechanistic perspective

Notes

SHORT TALK

Coupling of ribosome biogenesis and translation initiation in human mitochondria

Anna Franziska Finke*, Marleen Heinrichs*, Shintaro Aibara, Angelique Krempler, Angela Boshnakovska, Peter Rehling, Hauke Hillen, Ricarda Richter-Dennerlein,
*The authors declared to contribute equally
Department of Cellular Biochemistry, University Medical Center Göttingen, Göttingen, Germany Research Group Structure and Function of Molecular Machines, Max Planck Institute for Multidisciplinary Sciences, Göttingen, Germany Department of Molecular Biology, Max Planck Institute for Multidisciplinary Sciences, Göttingen, Germany

Biogenesis of mitoribosomes requires dedicated chaperones, RNA-modifying enzymes, and GTPases. Defects in this process cause severe mitochondriopathies, yet the molecular mechanisms remain poorly understood.
Here, we characterize late-step assembly states of the small mitoribosomal subunit (mtSSU) by combining genetic perturbation and mutagenesis analysis with biochemical and structural approaches. Single-particle cryo-EM analysis of native mtSSU biogenesis intermediates isolated via a FLAG-tagged variant of the GTPase MTG3 reveals three distinct assembly states, which show how factors cooperate to mature the 12S rRNA.
In addition, we observe four distinct primed initiation mtSSU states with an incompletely matured rRNA, suggesting that biogenesis and translation initiation are not mutually exclusive processes but can occur simultaneously.
Together, these results provide insights into mtSSU biogenesis and suggest a functional coupling between ribosome biogenesis and translation initiation in human mitochondria.

Notes

Machines acting on DNA and RNA, a molecular mechanistic perspective

Notes

SHORT TALK

Structural basis of de novo chromatin assembly during DNA replication

Jolijn Govers[1], Charlotte M. Delvaux de Fenffe[1], Fabian Gruss[1], Clément Rouillon[1], Pascal Albanese[2], Richard A. Scheltema[2], Juliette Fedry[2,3], Friedrich Förster[2], Francesca Mattiroli[1]

1. Hubrecht Institute-KNAW, Utrecht, The Netherlands,
2. Utrecht University, Utrecht, The Netherlands
3. MRC Laboratory of Molecular Biology, Cambridge, United Kingdom

During S-phase, replication of DNA and its organization into chromatin are tightly coordinated by PCNA. Yet, how the key chromatin assembly machinery, CAF-1, interacts with PCNA remains largely unknown. Here, we use cryo-electron microscopy and biochemical reconstitutions to resolve the structure of the yeast CAF-1–histone–PCNA complex on DNA. Our findings reveal that two asymmetric CAF-1 complexes bind to the trimeric PCNA ring. Beyond the well-characterized PIP-dependent interaction, we find that each CAF-1 engages PCNA through a flexible interface, that positions the histone-binding domain on the back of the PCNA ring. This distinct binding mode enhances PCNA stability on DNA, reinforcing its role in chromatin replication. In this way, the CAF-1 complexes envelop the PCNA ring, explaining the inhibitory effect observed on DNA polymerase activity. We demonstrate that this interaction is critical for heterochromatin integrity in cells. This work provides fundamental insights into the vital mechanisms that control chromatin assembly during DNA replication

Machines acting on DNA and RNA, a molecular mechanistic perspective

Notes

Machines acting on DNA and RNA, a molecular mechanistic perspective

Notes

cnio - CaixaResearch
FRONTIERS
MEETINGS

Madrid 28th – 30th May 2025

Machines acting on DNA and RNA, a molecular mechanistic perspective

Friday May 30th 2025

Session #6
Translation

Chairperson: ***Alessandro Costa***

Machines acting on DNA and RNA, a molecular mechanistic perspective

Cryo-electron microscopy of actively translating ribosomes

Christian M. T. Spahn
Institute of Medical Physics and Biophysics
Universitätsmedizin Berlin
Germany

At the heart of numerous biological processes are complex macromolecular assemblies, such as the ribosome. Throughout its functional cycles, the ribosome interacts with numerous ligands and factors, with these interactions driven and regulated by various conformational modes of the transiently formed intermediates. Structure determination of dynamic macromolecular machines and elucidating their conformational landscapes are fundamental tasks of structural biology. Traditionally, structural studies of functional ribosomal complexes rely on *in vitro* assembled complexes and biochemical means to stall ribosomes in defined states. However, such artificial stalling can introduce ambiguity, as it may fail to fully capture the natural state of the complex and could instead yield off-pathway intermediates rather than true physiological states. To overcome this limitation and solve the structures of native transient intermediates, we adopt a novel strategy: visualizing actively translating ribosomes. Our approach is based on the reactivation of *ex-vivo*-derived E. *coli* polysomes in the PURE *in vitro* translation system followed by multiparticle cryo-EM1. This enables comprehensive visualization of the translation elongation cycle and allows the study of rapid processes—such as GTPase factor-dependent reactions and peptidyl-transfer/accommodation—under near-native conditions, without artificially stalling the system using antibiotics or non-cleavable GTP *analogues.*

1. Flügel, T. et al. Transient disome complex formation in native polysomes during ongoing protein synthesis captured by cryo-EM. Nature communications 15, 1756 (2024).

Institut für Medizinische Physik und Biophysik, Charité – Universitätsmedizin Berlin, Charitéplatz 1, 10117 Berlin, Germany

Machines acting on DNA and RNA, a molecular mechanistic perspective

Notes

Machines acting on DNA and RNA, a molecular mechanistic perspective

Notes

SHORT TALK

Mechanisms to Create Specialized Ribosomes in Bacteria

Joaquin Ortega[1,2,*], Dominic Arpin[1,2], Bappaditya Roy[3,4], Fawwaz M. Naeem[4,5], Zakkary A. McNutt[4,5], Michael Brandt[4,5], Kurt L. Fredrick[3,4,5].

1. Department of Anatomy and Cell Biology, McGill University, Montreal, Quebec H3A 0C7, Canada.
2. Centre for Structural Biology, McGill University, Montreal, Quebec H3G 0B1, Canada.
3. Department of Microbiology, The Ohio State University, Columbus, OH 43210, USA.
4. Center for RNA Biology, The Ohio State University, Columbus, OH 43210, USA.
5. Ohio State Biochemistry Program, The Ohio State University, Columbus, OH 43210, USA.

It has been accepted for decades that all ribosomes in bacterial cells are functionally identical. However, various groups have recently shown that a single bacterial cell can contain multiple ribosome populations with different rRNA sequences or rprotein compositions. What remains unknown is whether the altered composition results in 'specialized' ribosomes that are functionally distinct. Our recent work has suggested the first mechanism by which bacteria create 'specialized' ribosomes that differ functionally. Sequencing studies revealed that most of the mRNAs in Flavobacterium johnsoniae (Fjo), a model organism for Bacteroidia, lack Shine- Dalgarno (SD) sequences. However, Fjo maintains a functional anti-Shine-Dalgarno (ASD) sequence in ribosomes, which is occluded by interactions with specific rproteins.

Ribosomes depleted of these proteins become 'specialized' and exploit the unlocking of the functional ASD sequence to enhance the translation of those particular mRNAs containing SD sequences. This shift in the translation pattern redirects translation to specific mRNAs, ultimately resulting in the production of certain proteins that allow bacteria to adapt and thrive under unfavorable conditions. Our recent results provide the first evidence, to our knowledge, for the existence of 'specialized' ribosomes that are functionally different in bacteria.

Notes

Machines acting on DNA and RNA, a molecular mechanistic perspective

Notes

Where to start: The Dynamic Adventure of Identifying Start Condons in mRNAs

Israel S. Fernández
Biofisika Institute (CSIC-UPV/EHU), Spain

During the process of translation, ribosomes produce proteins decoding the information stored in messenger RNAs (mRNAs). The linear nature of the message to decode requires the correct identification of the reading frame on the mRNAs. The identification of the first triplet (the mRNA start codon) and the delivery of the first aminoacyl-tRNA to this codon is an essential milestone to achieve for the successful production of functional proteins. The overall endeavor, termed translation initiation, is sophisticated, involving the small ribosomal subunit as well as dozens of protein factors and a dedicated aminoacyl-tRNA (Met-tRNAiMet). Single-particle CryoEM methods have revealed mechanistic insights on both, the overall architecture as well as the dynamics of the initiation process, allowing the formulation of a comprehensive model for how ribosomes search and find initiation codons. In this context, we will describe our studies focusing on the differences and similarities between canonical initiation and viral initiation with the aim to underline common basic principles within a mechanistic outlook.

Machines acting on DNA and RNA, a molecular mechanistic perspective

Notes

Machines acting on DNA and RNA, a molecular mechanistic perspective

Notes

From RNA to Membrane: Mitochondrial Ribosomes at Work

Alexey Amunts
University of Münster,
EMBO Young Investigator Lecture
Germany

The mitoribosome translates mitochondrial mRNAs and regulates energy production, a hallmark of eukaryotic life. We present cryo-EM analyses of mitoribosome assembly intermediates, mRNA binding, and nascent polypeptide delivery to the membrane. To study the assembly mechanism, we identified small mitoribosomal subunit intermediates in complex with regulatory factors, elucidating how step-specific factors establish the catalytic mitoribosome. Once mitoribosomal subunits are mature, mRNA delivery is then facilitated by the helical repeat factor LRPPRC, which forms a stable complex with SLIRP, stabilizing mRNAs co-transcriptionally and linking the gene expression system. Finally, nascent polypeptides are delivered to the mitochondrial inner membrane, and we report the mitoribosome structure bound to the insertase OXA1, explaining the coupling of protein synthesis to membrane delivery. Along with the identification of functional cofactors and specific rRNA modifications, the data illuminate the principal components responsible for mitochondrial translation and its regulation.

Machines acting on DNA and RNA, a molecular mechanistic perspective

Notes

Machines acting on DNA and RNA, a molecular mechanistic perspective

Notes

cnio - CaixaResearch
FRONTIERS
MEETINGS

Madrid 28th – 30th May 2025

Machines acting on DNA and RNA, a molecular mechanistic perspective

Organisers & Speakers' Biographies

cnio stop cancer

Dr. Oscar Llorca

Structural Biology Programme Director
Macromolecular Complexes in DNA Damage Response Group
Spanish National Cancer Research Centre (CNIO)
Madrid, Spain

Oscar Llorca obtained his PhD in 1996 from the Autonomous University of Madrid. Then, he performed his postdoctoral studies at the Institute of Cancer Research (ICR), London, UK, with a Marie Skłodowska-Curie fellowship. In 2002 he moved back to Spain as a Group leader at the Centre for Biological Research (CIB), CSIC, Madrid. Since June 2017, he has been leading the Macromolecular Complexes in DNA Damage Response Group at CNIO, and he is also a Director of the Structural Biology Programme. His main interest is the use of cryo-electron microscopy to study large macromolecular complexes in the DNA damage response, and molecular chaperones. He has published more than 130 articles in peer-reviewed journals, including Nature, Science, Nature SMB, Nature Communications, Molecular Cell, Cell reports, The EMBO Journal, Proceedings of the National Academy of Sciences (PNAS), and several others.

ORGANISERS & SPEAKERS' BIOGRAPHIES

Dr. Rafael Fernández Leiro

Genome Integrity and Structural Biology Group
Structural Biology Programme
Spanish National Cancer Research Centre (CNIO)
Madrid, Spain

Since 2017, I have led the Genome Integrity and Structural Biology Group at CNIO, where I helped revamp the Structural Biology Programme, implementing high-resolution cryo-EM and AI-based tools. My group investigates dynamic protein complexes involved in DNA replication and maintenance, focusing on cancer relevance. We apply multidisciplinary approaches that integrate cryo-EM, biochemical and biophysical assays. Prior to CNIO, I trained in cryo-EM and structural biology at the MRC-LMB in Cambridge, gaining experience in method development and DNA replication and repair. These experiences have shaped my current efforts to dissect genome maintenance mechanisms at the molecular level.

Machines acting on DNA and RNA, a molecular mechanistic perspective

Dr. Eva Nogales

University of California, Berkeley
Dept. of Molecular & Cell Biology
Berkeley, CA US

She is a Distinguished Professor in Molecular and Cell Biology at the University of California, Berkeley, a Howard Hughes Medical Institute Investigator, and a Senior Faculty Scientist at LBNL. Nogales is a member of the National Academy of Sciences of the USA, the American Academy of Arts & Sciences, the Royal Academy of Sciences of Spain and of EMBO. In 2023 she received the Shaw Prize in Life Science and Medicine. Her lab uses cryo-EM to describe the structure, dynamics and interactions of large biological assemblies, including microtubules and transcription and epigenetic complexes.

ORGANISERS & SPEAKERS' BIOGRAPHIES

Dr. Alessandro Vannini

Head of Structural Biology Research Centre
Human Technopole, Milan, Italy

Prof. Alessandro Vannini is the Head of the Center for Structural Biology at Human Technopole, Milan. He received a PhD in Biochemistry and Molecular Biology from the Faculty of Medicine, Tor Vergata University of Rome in 2006 and was a postdioctoral fellow at the Gene Center of the Ludwig-Maximilians-Universität (LMU) in Munich. In 2012, he started his independent group at the Institute of Cancer Research (ICR) in London, first as a Team Leader and then as deputy head of the Division of Structural Biology and was awarded the title of Professor of Integrative Structural Biology. Throughout his career, he received numerous awards and honors: EMBO member (2023), EMBO Young Investigator (2016), Wellcome Trust Investigator (2016), CR-UK Program Foundation Award (2016), New Investigator Award Biotechnology and Biological Sciences Research Council (2013), Römer-Prize Ludwig-Maximilians- Universität (LMU), (2010), Marie Curie Intra-European fellowship (2007) and European Molecular Biology Organization (EMBO) long-term fellowship (2006).

Machines acting on DNA and RNA, a molecular mechanistic perspective

Dr. Nicole M. Hoitsma

Biochemistry
University of Colorado Boulder
Boulder, US

Dr. Hoitsma received her B.A. from South Dakota State University, where she developed an interest in molecular mechanisms underlying genome maintenance. She completed her Ph.D. in Biochemistry and Molecular Biology at the University of Kansas Medical Center, studying the structural biology of DNA damage repair pathways. Currently, as an HHMI Fellow of the Damon Runyon Cancer Research Foundation in Karolin Luger's lab at the University of Colorado Boulder, her postdoctoral research explores how chromatin structure and remodeling influence the cellular response to DNA damage, with the goal of understanding how these processes maintain genome integrity and contribute to disease when dysregulated.

ORGANISERS & SPEAKERS' BIOGRAPHIES

Siddhant U. Jain, Ph.D.

Dana-Farber Cancer Institute
Harvard Medical School
Howard Hughes Medical Institute
Boston, US

Dr. Sid Jain is a research fellow at Dana-Farber Cancer Institute. Dr. Jain is interested in understanding the biochemical mechanisms by which perturbations in chromatin modifying enzymes, including members of the Trithorax and Polycomb group complexes, drive human diseases including cancers.

Machines acting on DNA and RNA, a molecular mechanistic perspective

Dr. Yuan He

Thomas C. Jenkins Department of Biophysics
Johns Hopkins University
Baltimore, US

Yuan He earned his PhD in Biochemistry and Biophysics at Northwestern University, then headed west to UC Berkeley for post-doctoral work in structural biology with Eva Nogales. He returned to Northwestern to launch his own lab, exploring how large, multi-subunit complexes carry out DNA-centered tasks. In 2024, He moved to Johns Hopkins University, where he is now the Bloomberg Distinguished Professor of Structural Biophysics and Chromatin Biology, continuing to dissect how cells read and repair the genome.

ORGANISERS & SPEAKERS' BIOGRAPHIES

Dr. Wei Yang

Laboratory of Molecular Biology
National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)
National Institutes of Health
Bethesda, USA

Wei Yang is a Distinguished Investigator at the U.S. National Institutes of Health, where she has worked since 1995. She began her undergraduate studies in biochemistry at Fudan University in Shanghai, China and later transferred to the State University of New York at Stony Brook, where she received a B.A. degree. She completed her Ph.D. at Columbia University under the guidance of Wayne Hendrickson and conducted postdoctoral research with Tom Steitz at Yale University. Her research focuses on using structural and biochemical methods to elucidate the molecular mechanisms underlying DNA replication, repair, and recombination, particularly in ATP-mediated and Mg^{2+}-dependent pathways.

Machines acting on DNA and RNA, a molecular mechanistic perspective

Dr. Elizabeth (Liz) Kellogg

Department of Structural Biology
Jude Children's Research Hospital
Memphis, US

Elizabeth (Liz) Kellogg started her independent career in 2019 and has already made significant contributions to the transposon field. Her research is aimed at understanding how transposons reshape genomes and how to repurpose them as genome editing tools. Liz's group has focused on the molecular mechanisms of CRISPR-associated transposons (CASTs), RNA-guided mobile genetic elements that hold enormous potential for gene replacement therapy. The Kellogg lab combines cryo-electron microscopy, biochemistry, single-molecule and genetic approaches to investigate how the transposon DNA is recruited and inserted at a CRISPR-defined target site and how this complex process is exquisitely regulated, spatially and temporally. Liz's work has advanced our understanding of the mechanisms of RNA-guided DNA transposition, paving the way to develop new genome engineering tools. Her talent and scientific accomplishments have been recognized with multiple honors, including her selection as Pew Biomedical Scholar and Rita Allen Scholar in 2021, the Robert N Noyce Endowed Professorship in 2021, and the 2023 Margaret Oakley Dayhoff Award from the Biophysical Society in 2023.

ORGANISERS & SPEAKERS' BIOGRAPHIES

Machines acting on DNA and RNA, a molecular mechanistic perspective

Dr. Jun-Jie Gogo Liu

Associate Professor
School of Life Sciences
Tsinghua University
Beijing, China

2011-2016, Ph.D., School of Life Sciences, Tsinghua University
2016-2020, Postdoctoral Fellow, Lawrence Berkeley National Laboratory and UC Berkeley
09/2020-11/2023, Assistant professor, School of Life Sciences, Tsinghua University
12/2023 to date, Associate professor, School of Life Sciences, Tsinghua University
Gogo Liu's group has extensively employed multidisciplinary approaches to investigate and engineer RNA-associated machineries capable of nucleic acid manipulation. They have successfully established several gene editing systems, including CRISPR-Casπ and the HYER ribozyme, which cleaves DNA through hydrolysis. Additionally, the group has identified proCRISPR factors that enhance Cas9's DNA targeting efficiency, elucidated the regulatory role of structural RNAs in ensuring precise retrotransposon transposition, and developed a retrotransposon-derived tool for large-scale gene fragment insertion.

Lab Page: https://gogolab.life.tsinghua.edu.cn

Dr. Gina Buchel

Department of Molecular, Cellular, and Developmental Biology
Yale University
New Haven, US

My research interests lie in characterizing protein machines that interact with RNA and DNA on the molecular and mechanistic level. During my Ph.D. training, I investigated the roles of two molecular machines integral to human mitochondrial replication, DNA Polymerase Gamma and the replicative helicase TWINKLE, in maintaining mitochondrial DNA fidelity. Currently, I am a post-doctoral fellow in Dr. Anna Marie Pyle's laboratory at Yale University, where I study the mechanism by which the innate immune modulator MDA5 functions in an antiviral response and how its activity is regulated within the cell.

ORGANISERS & SPEAKERS' BIOGRAPHIES

Dr. Lori A Passmore

MRC Laboratory of Molecular Biology
Cambridge, UK

Lori A Passmore is a Group Leader and Joint Head of Structural Studies at the MRC Laboratory of Molecular Biology in Cambridge UK. She uses an integrated approach combining structural, biochemical and functional studies, aiming to reconstitute multi-protein complexes and their activities, and to determine their high-resolution structures to understand their mechanisms. She works on protein complexes that add and remove poly(A) tails from mRNAs and complexes involved in DNA repair. She was elected a member of EMBO in 2018, received the RNA Society's Inaugural Elisa Izaurralde Award in 2020 and was elected a Fellow of the Royal Society in 2023.

Machines acting on DNA and RNA, a molecular mechanistic perspective

Dr. Elena Conti

Max Planck Institute of Biochemistry
Structural Cell Biology Department
Munich, Germany

Elena Conti is a biochemist renowned who has made important contributions to understanding RNA degradation, export, and surveillance mechanisms. She earned her PhD from Imperial College (London) and completed postdoctoral training at The Rockefeller University (New York). Since 2007, Conti has served as a director at the Max Planck Institute of Biochemistry (Munich). Her work combines biochemistry and structural to elucidate the function of protein-RNA machineries. A member of several prestigious scientific academies, such as EMBO, the Roral Society (UK) and the Lincei (IT), Conti has received numerous awards, including the Louis-Jeantet Prize for Medicine, the Gregori Aminoff Prize.

ORGANISERS & SPEAKERS' BIOGRAPHIES

Dr. Xiaodong Zhang

Professor of Macromolecular Structure and Function
Imperial College London, https://profiles.imperial.ac.uk/xiaodong.zhang
& The Francis Crick Institute https://www.crick.ac.uk/research/labs/xiaodong-zhang

Xiaodong Zhang studied Nuclear Physics in Peking University, China, and then pursued a PhD in Physics at Stony Brook University in the United States, where she developed X-ray microscopy with chemical contrast. After earning her PhD, she transitioned to structural biology, undertaking postdoctoral training in X-ray crystallography at Harvard University. In 1997, Dr. Zhang joined Imperial Cancer Research Fund (now part of Francis Crick Institute) as a postdoctoral researcher. She become a lecturer at Imperial College London in 2001, was promoted to Reader in 2005 and appointed Professor of Macromolecular Structure and Function in 2008. She served as Director of Imperial College Centre for Structural Biology between 2011 and 2014. In 2014, she moved to the Faculty of Medicine at Imperial College London to help establishing the Section of Structural Biology, which fosters the integration of structural biology with medical research at Imperial College London. She has recently been seconded to the Francis Crick Institute. Dr Zhang's current research focuses on understanding the mechanisms of molecular machines, particularly those involved in DNA processing, such as the transcription machinery and DNA damage signalling and repair. She is the recipient of the Royal Society Wolfson Research Merit Award, the Wellcome Trust Investigator Award and the Wellcome Trust Discovery Award. Dr. Zhang is an elected Fellow of the Royal Society of Biology (UK, 2011), elected member of the European Molecular Biology Organisation (EMBO, 2016), and an elected Fellow of the Royal Society (2024).

Machines acting on DNA and RNA, a molecular mechanistic perspective

Dr. Alessandro Costa

Macromolecular Machines Laboratory
The Francis Crick Institute
London, UK

Alessandro Costa studied Biotechnology at the University of Padova and received his PhD from Imperial College London in 2008, having studied archaeal helicases under the supervision of Silvia Onesti. He then moved to Steve Bell's group at Oxford University to investigate archaeal chromosome replication and joined UC Berkeley as an EMBO fellow, where he determined the first structure of an active eukaryotic replicative helicase with James Berger. In 2012, he established his own group at the CRUK Clare Hall Laboratories and later moved to the Francis Crick Institute in London. Alessandro was elected a member of EMBO in 2024.

Dr. Leemor Joshua-Tor

Howard Hughes Medical Institute
Cold Spring Harbor Laboratory
New York, US

Leemor Joshua-Tor, Ph.D., is a Howard Hughes Medical Institute Investigator and W.M. Keck Professor of Structural Biology at Cold Spring Harbor Laboratory. She is also the Director of Research at CSHL. She earned her Ph.D. at the Weizmann Institute of Science and was a Jane Coffin Childs postdoctoral fellow at Caltech prior to joining the CSHL faculty. Leemor Joshua-Tor's laboratory studies the molecular basis of nucleic acid regulatory processes, RNA interference (RNAi), and DNA replication initiation in particular.

Dr. Meindert Lamers

Co-director NeCEN & Associate professor Leiden University Medical Center (LUMC)
Institute of Biology Leiden
The Netherlands

He graduated in Medical Biology at the University of Amsterdam. After that, I did my PhD research at the Netherlands Cancer Institute under the supervision of Titia Sixma, working on DNA mismatch repair. Following my PhD, I did a postdoc at UC Berkeley under the supervision of John Kuriyan where I worked on the bacterial DNA polymerase Pol III. In 2009 I set up my own research group at the Laboratory of Molecular Biology in Cambridge, UK. In 2017 I moved to the Leiden University Medical Center. Since 2019 I am director of the Netherlands Center of Electron Nanoscopy (NeCEN).

ORGANISERS & SPEAKERS' BIOGRAPHIES

Dr. Christian M.T. Spahn

Institute of Medical Physics and Biophysics
Universitätsmedizin Berlin
Germany

Christian M.T. Spahn is Professor of Biophysics and Director of the Institute for Medical Physics and Biophysics at Charité – Universitätsmedizin Berlin. He studied Biochemistry at Freie Universität Berlin and earned his PhD in 1996 under Knud Nierhaus at the Max Planck Institute for Molecular Genetics. Following postdoctoral research with Joachim Frank at HHMI/Wadsworth Center, Albany, he established his lab at Charité in 2002. A member of EMBO and the Leopoldina, his research focuses on cryo-EM of macromolecular machines, particularly the structure, function, regulation, and assembly of ribosomes.

Machines acting on DNA and RNA, a molecular mechanistic perspective

Dr. Israel S. Fernández
Biofisika Institute (CSIC-UPV/EHU), Spain

Dr. Israel S. Fernández is a structural biologist with a trajectory focused on RNA. Along my scientific journey, I first made use of X-ray crystallography techniques and later single-particle electron cryo-microscopy (CryoEM) to visualize RNA molecules in action. Using these technical approaches, I made contributions to understand mechanistically two key biological phenomena involving RNA molecules: how ribosomes place the first aminoacyl-tRNA in the initiation codon of the messenger RNA (a process termed translation initiation) and more recently, I provided structural knowledge to understand a family of transposons that uses CRISPR-derived RNA molecules to guide the DNA mobilization process to the target location.

Dr. Alexey Amunts
University of Münster,
Germany

Alexey Amunts earned his PhD from Tel Aviv University for his work on plant Photosystem I in the laboratory of Nathan Nelson in 2010. He did a postdoc at the MRC Laboratory of Molecular Biology in Cambridge, focusing on cryo-EM studies of the mitoribosome with Venki Ramakrishnan. In 2016, he established an independent group at Stockholm University, focusing on mechanisms of mitochondrial translation and bioenergetics. The group investigates protein synthesis and energy production at the molecular and cellular level, examining how these fundamental processes are affected by natural selection and disease. He has been awarded the EMBO Young Investigator Award and ERC grant. Currently, Alexey Amunts serves as a Humboldt Fellow at Max Planck Institute for Physiology in Dortmund.

Machines acting on DNA and RNA, a molecular mechanistic perspective

cnio - CaixaResearch FRONTIERS MEETINGS

Madrid 28th – 30th May 2025

Machines acting on DNA and RNA, a molecular mechanistic perspective

Wednesday May 28th 2025

Poster Session group 1

cnio stop cancer

01

Chromosome-wide Gene Regulation by DNA Loop Extrusion and Liquid-Liquid Phase Separation

Gurumoorthy Amudhan, Antonio Valdes, Iris Sutter, Anja Hämmerli, Dario Bernasconi, Sinem Erkan, Jennifer Semple, Brigitta Wilde, Markus Hassler, Peter Meister & Christian H. Haering

Department of Biochemistry and Cell Biology, Julius Maximilian University of Würzburg, 97074 Würzburg, Germany
Cell Fate and Nuclear Organization; University of Bern; Bern; CH-3012; Switzerland

Condensins are hetero-pentameric protein complexes of the Structural Maintenance of Chromosomes (SMC) family that act as ATP-dependent DNAlooping motors to fold chromatin into rod-shaped chromosomes during mitosis and meiosis. The two distinct condensin complexes in metazoans termed condensin I or II carry out non-overlapping functions in structuring chromosome architecture. In Caenorhabditis elegans, a specialized paralog of condensin I, known as condensin IDC, collaborates with a set of Sex Determination and Dosage Compensation (SDC) proteins to downregulate gene expression from both X chromosomes in hermaphrodite animals, thereby balancing expression with the single X chromosome in males. The mechanisms that guide condensin IDC specifically to the X chromosome and mediate its repressive function remain incompletely understood.

We show that in the absence of key SDC components, condensin IDC binds nonspecifically chromosomal DNA, both in vivo and in vitro. We identify a direct physical interaction between condensin IDC and an SDC protein, resolve the

Other Information:

structure of this interface via cryo-EM, and demonstrate the importance of the protein-protein interface by mutation.

Moreover, we provide evidence that a large intrinsically disordered region of the interacting SDC protein drives the formation of membrane-less 'SDC bodies'through nucleic acid-dependent liquid-liquid phase separation. Based on our finding of a DNA loop-extruding activity of condensin IDC, which mirrors that of canonical SMC complexes, we propose that its motor function is repurposed for chromosome-wide gene repression by targeted recruitment to the X chromosome and active extrusion of DNA through a phase-separated compartment. Finally, we present near-atomic resolution insights into the condensin IDC ATPase core, shedding light on its molecular mechanism of action.

02

Studies on the molecular interplay between p97 and three of its protein adaptors in the DNA-protein crosslink repair

Maša Andoljšek, Primož Bembič, Matic Kisovec, Marjetka Podobnik

Department of Molecular Biology and Nanobiotechnology, National Institute of Chemistry, Hajdrihova ulica 19, Ljubljana, Slovenia

DNA-protein crosslinks (DPCs) are common genomic lesions that hinder DNA replication, transcription, chromatin remodelling, recombination and repair. DPCs are formed when proteins crosslink to DNA, upon exposure to inducers or when DNA transaction enzymes are trapped. Impaired DNA-Protein Crosslink Repair (DPCR) is linked to cancer, ageing and neurodegeneration. About a decade ago, the yeast protease Wss1 was found to remove DPCs, and in metazoans a similar enzyme, the protease SPRTN (SprT-like N-terminal domain), was found to remove DPCs during replication[1,2].

The ATPase p97 (also known as VCP) acts together with its adaptors Ufd1 and Npl4 in various cellular processes. Ufd1 and Npl4 form a dimeric adaptor complex (UN) that recruits specific ubiquitinated substrates to p97 and facilitates their unfolding by p97 and consequently their degradation by cellular proteases[3]. While SPRTN has been shown to be able to cleave the loosely folded DPCs by itself, it has been hypothesised that compact DPCs can only be cleaved by SPRTN if they have been previously unfolded by p97 with the help of the UN complex[4].

The aim of our study is to understand the molecular interplay between p97 and its three adaptor proteins, Ufd1, Npl4 and SPRTN. I will present the molecular characterisation of these proteins using different biochemical and biophysical approaches, as well as their interactions analysed by size exclusion chromatography (SEC), microscale thermophoresis (MST), mass photometry (MP) and cryo electron microscopy (Cryo-EM).

Other Information:

Literature:
1. Stingele, J. et al. Mechanisms of DNA-protein crosslink repair. Nat. Rev. Mol. Cell. Bio. 2017.
2. Meyer HH, et al. Direct binding of ubiquitin conjugates by the mammalian p97 adaptor complexes, p47 and Ufd1-Npl4. EMBO J. 2002.
3. Braxton JR, Southworth DR. Structural insights of the p97/VCP AAA+ ATPase: How adapter interactions coordinate diverse cellular functionality. J. Biol. Chem. 2023.
4. Kröning A, et al. Ubiquitin-directed AAA+ ATPase p97/VCP unfolds stable proteins crosslinked to DNA for proteolysis by SPRTN. J. Biol. Chem. 2022.

03

Molecular basis for the initiation of primer synthesis by Primase-Polymerases

Aran-Guiu, X.[1], Li, A.W.H.[1], Zabrady, K.[1,2], Bainbridge, L.[1,3], & Doherty, A.J.[1]

1. Genome Damage Stability Centre, School of Life Sciences, University of Sussex, Brighton, UK.
2. Central European Institute of Technology, Masaryk University, Brno, Czech Republic.
3. Japanese Foundation for Cancer Research, Tokyo, Japan

Primase-polymerase (Prim-Pol) replicases are found across all domains of life and play essential roles in DNA replication, repair, damage tolerance and adaptive immunity [1]. Despite their biological importance, the molecular mechanism by which Prim-Pols initiate primer synthesis is unclear. To understand this priming mechanism, we elucidated the crystal structure of a primase initiation complex of a CRISPR-associated Prim-Pol (CAPP) [2]. The structure revealed how this primase accommodates single-stranded DNA (ssDNA), incoming nucleotides and metal ions within its active site to catalyse the first phosphodiester bond.
Structural comparisons between CAPP, Primase 1, and PrimPol reveal a remarkable conservation of their active site architectures, suggesting a unified catalytic mechanism for primer initiation [2].
Next, we studied human PrimPol to determine if it utilises a similar mechanism to reprime DNA synthesis downstream of obstacles that stall genome duplication [3]. The core human Prim- Pol domain alone, without its zinc finger domain, is sufficient to catalyse de novo primer synthesis [4]. Biochemical and biophysical studies revealed that PrimPol exhibits a defined template sequence specificity and a preference to initiate primers using purine nucleotides [2, 5]. Furthermore, our studies revealed an ordered 'choreography' during primer initiation and identified key active site residues critical for coordinating the template DNA and nucleotides. These studies identified that a precise network of molecular interactions and metal ion coordination drives efficient primer synthesis. Notably, PrimPol's priming mechanism closely mirrors that of CAPP, providing strong evidence for a conserved primer initiation process.
Together, these findings provide a comprehensive molecular model explaining how PrimPol synthesises primers from scratch. It reveals how the compact catalytic core, without auxiliary domains, is sufficient to perform the critical first step of primer synthesis. These studies refine our understanding of human PrimPol function and establish that this mechanism of primer synthesis is conserved across all domains of life.

1. Guilliam, T.A., et al. Nucleic Acids Res, 2015. 43(14): p. 6651-64.
2. Li, A.W.H., et al. Nature, 2022. 605(7911): p. 767-773.
3. Bainbridge, L.J., R. Teague, and A.J. Doherty. Nucleic Acids Res, 2021. 49(9): p. 4831-4847.
4. Keen, B.A., et al. Nucleic Acids Research, 2014. 42(9).
5. Bainbridge, L.J., K. Zabrady, and A.J. Doherty. J Mol Biol, 2023. 435(24): p. 168338.

04

Structural and functional studies of human endogenous retroviruses (hERVs)

Ane Barrena-Martin, Sandra Fuertes Ruiz, Ian Gonzalez Casado, Guillermo Abascal- Palacios

Instituto Biofisika (UPV/EHU-CSIC)

For decades, the human genome has been considered a static element whose composition and architecture remained unaltered after embryogenic development was completed. This lasting idea has been recently challenged through the development of very precise tools that allow us to study the genomic landscape and its interacting networks. Thanks to new techniques such as high-throughput sequencing or CHIP-seq, among others, now it is widely accepted that human chromosomes are much more complex than previously expected. One of the main discoveries was that transposable elements, which had been traditionally considered "junk" regions of the genome, are very abundant and their activity is directly associated with the development of several human diseases. Due to this historical misconception, the study of human retrotransposons, and particularly the analysis of the molecular basis of their activity, has not been thoroughly accomplished.
This project aims to fulfil some of those questions. Specifically, it will focus on deciphering the underlying mechanism of human endogenous retroviruses (hERVs) activity, determining the architecture of integration complexes and the association to host cellular factors, which are linked to disease. To achieve these objectives, the project will employ a multidisciplinary approach, which includes structural biology, biochemistry, biophysics and microbiology tools.

05

Characterization of the molecular interactions between the DNA-protein crosslink repair partners p97 and SPRTN

Primož Bembič[1], Matic Kisovec[1], Andreja Kežar[1], Maksimiljan Adamek[1], Marta Popović[2], Marjetka Podobnik[1]

1. Departement of Molecular Biology and Nanobiotechnology, National Institute of Chemistry, Hajdrihova ulica 19, Ljubljana, Slovenia
2. Laboratory for molecular ecotoxicology, Ruđer Bošković Institute, Bijenička cesta 54, Zagreb, Croatia

DNA-protein crosslinks (DPCs) are DNA lesions that occur when a protein is irreversibly covalently bound to DNA. DPCs have detrimental effects on the organism, including cancer, premature aging and neurodegenerative diseases. Due to their bulky nature, DPCs interfere with all DNA transactions such as replication, transcription and repair, making DPC repair an essential cellular pathway [1].
DPC repair involves several mechanisms that remain largely unexplored. We are investigating two human proteins involved in the DPC repair process: a DNAdependent metalloprotease called SPRTN and a hexameric AAA+ unfoldase p97. In contrast to the structured N-terminal protease domain of SPRTN, the C-terminal part of the protein is intrinsically disordered. There are many interaction motifs within this unstructured region. One of them is the SHP motif, which is thought to be the main interaction motif between SPRTN and p97, but is also used by many other proteins that bind to p97 [2, 3].
We have investigated the molecular interactions between p97 and SPRTN using various biochemical and biophysical approaches such as pull-down assays, size exclusion chromatography (SEC), microscale thermophoresis (MST) and isothermal titration calorimetry (ITC). Due to the low sample consumption, fast assay set-up and optimization, the first interaction experiments were carried out using MST. The results of the MST experiments were then confirmed with ITC experiments and showed that SPRTN binds to p97 with an affinity in the low micromolar range.
We also performed a structural analysis of the p97:SPRTN complex using cryoelectron microscopy, which provided high-resolution evidence for the interaction between these two proteins through the SHP motif of SPRTN.

Other Information:
References:
[1] Aguilera, A. & Gómez-González, B. Genome instability: A mechanistic view of its causes and consequences. Nature Reviews Genetics vol. 9 204–217 (2008)
[2] Davis, E. J. et al. DVC1 (C1orf124) recruits the p97 protein segregase to sites of DNA damage. Nat. Struct. Mol. Biol. 19, 1093–1100 (2012)
[3] Mosbech, A. et al. DVC1 (C1orf124) is a DNA damage-targeting p97 adaptor that promotes ubiquitin-dependent responses to replication blocks. Nat. Struct. Mol. Biol. 19, 1084–1092 (2012)

06

The A, B, C, D's of DNA polymerase fidelity

Leonardo Betancurt-Anzola, Killian O'Connel, Vladimir Potapov, Nathan Tanner, Ludovic Sauguet, Kelly M. Zatopek

1: Architecture and Dynamics of Biological Macromolecules, Institut Pasteur, Université Paris Cité, CNRS, UMR 3528, Paris, France.
2: New England Biolabs Inc., 240 County Road, Ipswich, MA 01938, USA.
3: New England Biolabs France, 5 Rue Henri Auguste Desbruères, 91000 Évry- Courcouronnes.
4: Sorbonne Université, Collège Doctoral, ED 515, Paris France.

DNA replication is a highly accurate process that requires a replicative DNA polymerase. These enzymes possess two catalytic activities: a selective DNAdependent DNA polymerase activity that incorporates deoxynucleotide triphosphates (dNTPs) and a proofreading/exonuclease activity that can remove incorrectly incorporated dNTPs. These two activities are essential for highly accurate, high fidelity DNA replication. Here, we utilize high-throughput sequencing to measure the fidelity of different families of replicative DNA polymerases. We focus our efforts on archaeal, Family B & D, and bacterial, Family A & C, replicative DNA polymerases to understand the contribution of the two catalytic activities for accurate genome replication. Using Pacific Bioscience Single- Molecule Real-Time (SMRT) sequencing, we measure the fidelity of wild type and exonuclease deficient versions of these polymerases. Despite a common hydrolysis mechanism, the contribution of the exonuclease domain of different replicative DNA polymerases varies, from increasing fidelity 2-fold in Family D DNA polymerases, to 15-fold in Family B DNA polymerases. Additionally, we observe vastly different error profiles for different DNA polymerase families.
We further explore the role of polymerase active site residues in dictating DNA polymerase error profiles and uncover a novel role for a bulky residue that prevents ribonucleotide incorporation, i.e. the steric gate, in modulating DNA polymerase fidelity. This study expands our understanding of the contributions and coordination of the polymerase and exonuclease active sites and the structural factors that govern DNA polymerase fidelity.

07

08

Structural and functional mechanisms of condensin II regulation by M18BP1

Alessandro Borsellini, Valentina Cecatiello, Joanna J. Andrecka and Alessandro Vannini

Human Technopole

Condensin II is a multi-subunit protein complex that, together with condensin I, cohesin, Top2A, and Kif4A, plays a central role in genome organization and chromosome condensation during mitosis in human cells. As a member of the structural maintenance of chromosomes (SMC) family, condensin II uses the energy
from ATP hydrolysis to extrude DNA loops, thereby compacting the genome into discrete mitotic chromosomes. Given its continuous presence in the nucleus throughout the cell cycle, condensin II activity must be tightly regulated to ensure that chromosome condensation occurs exclusively during mitosis. Our findings, in line with previous work, demonstrate that condensin II activity is inhibited by MCPH1 during interphase and activated by M18BP1 at the onset of mitosis.
To uncover the molecular basis of this regulation, we used cryo-electron microscopy (cryo-EM) to determine multiple structures of human condensin II at different stages of the ATPase cycle, both in the presence and absence of DNA and M18BP1. Complementing these structural studies, we employed dual-trap optical tweezers with fluorescence detection to investigate DNA binding properties and activity of condensin II complexes. These experiments revealed how M18BP1 enhances condensin II binding stability and promotes DNA compaction activity in an ATP-dependent manner. Together, our results provide mechanistic insights into how M18BP1 activates condensin II to drive mitotic chromosome assembly.

09

Plant BCL-DOMAIN HOMOLOG proteins play a conserved role in SWI/SNF complex stability

Joan Candela-Ferre[1], Jaime Pérez-Alemany[1], Borja Diego-Martin[1], Vijaya Pandey[2], James Wohlschlegel[2], Jorge Lozano-Juste[1], Javier Gallego-Bartolomé[1]

1. Instituto de Biología Molecular y Celular de Plantas, Consejo Superior de Investigaciones Científicas-Universitat Politècnica de València, Valencia 46022, Spain.
2. Department of Biological Chemistry, David Geffen School of Medicine, University of California, Los Angeles, CA 90095.

The SWItch/Sucrose Non-Fermenting (SWI/SNF) complexes are evolutionarily conserved, ATP-dependent chromatin remodelers crucial for multiple nuclear functions in eukaryotes. Recently, plant BCL-DOMAIN HOMOLOG (BDH) proteins were identified as shared subunits of all plant SWI/SNF complexes, significantly impacting chromatin accessibility and various developmental processes in Arabidopsis. In this study, we performed a comprehensive characterization of bdh mutants, revealing the role of BDH in hypocotyl cell elongation. Through detailed analysis of BDH domains, we identified a plant-specific N-terminal domain that facilitates the interaction between BDH and the rest of the complex.

Additionally, we uncovered the critical role of the BDH β-hairpin domain, which is phylogenetically related to mammalian BCL7 SWI/SNF subunits. While phylogenetic analyses did not identify BDH/BCL7 orthologs in fungi, structure prediction modeling demonstrated strong similarities between the SWI/SNF catalytic modules of plants, animals, and fungi and revealed the yeast Rtt102 protein as a structural homolog of BDH and BCL7. This finding is supported by the ability of Rtt102 to interact with the Arabidopsis catalytic module subunit ARP7 and partially rescue the bdh mutant phenotypes. Further experiments revealed that BDH promotes the stability of the ARP4-ARP7 heterodimer, leading to the partial destabilization of ARP4 in the SWI/SNF complexes. In summary, our study unveils the molecular function of BDH proteins in plant SWI/SNF complexes and suggests that β-hairpincontaining proteins are evolutionarily conserved subunits crucial for ARP heterodimer stability and SWI/SNF activity across eukaryotes.

10

Capturing the ultrastructure of PML nuclear bodies in human fibroblasts using cryo-CLEM, pFIB milling and cryo-ET

Arianna Raponi[1], Iain Harley[1], Benjamin G Davis[2,3], **Stephen D Carter[1]**

1. MRC-University of Glasgow Centre for Virus Research, Glasgow, United Kingdom, \
2. The Rosalind Franklin Institute, Harwell, UK. 3Department of Chemistry, University of Oxford, Oxford, OX1 3TA, UK.

Promyelocytic leukaemia (PML) nuclear bodies (NBs) are liquid-liquid phase separated sub-nuclear organelles that contain over 200 unique proteins underlying many diverse cellular processes. The assembly of PML NBs rely on the formation of higher-order complexes of PML and SUMO proteins to generate a phase separated shell surrounding a phase separated core. The PML shell, together with the core function to dynamically regulate abundance and modifications of "client" proteins inside the PML NB to ensure adaption to various cellular conditions, including multiple genome maintenance pathways such as the DNA damage response, DNA repair, and telomere homeostasis. To visualise the PML NB proteome in unprecedented detail we applied plasma focused ion beam (pFIB) milling guided by cryogenic correlated light and electron microscopy (cryo-CLEM), to gain access to PML-YFP NBs inside electron transparent lamella of human fibroblasts. We then captured PML-YFP NBs using cryo-electron tomography (cryo-ET) and performed template matching and sub-tomogram averaging to further interrogate the molecular landscape of PML-YFP NBs. Our cryo-tomograms of PML-YFP NB's revealed dramatic new structural insights into the unique architectures of the shell and core compartments. We independently confirmed our cryo-ET findings, by comparing super-resolution 3D-SIM images of PML-YFP NBs. This combined evidence suggests the shell is a roughly 50 nm ring composed mainly of large electron-dense complexes, whilst the core resembles a sphere with a much smoother texture. Unexpectedly, we also found within the core a densely packed region of proteins, we termed the inner-core. These findings advance our understanding of phase separation within the nucleus. Our ultimate goal is to digitally catalogue the proteome and ultrastructure of PML NBs under various conditions to interrogate how dysregulation of these compartments affects genome integrity and their abnormalities in disease.

11 Exploring the Shelterin Complex: organisation and key interactions

Ester Casajus-Pelegay, María Moreno-Morcillo, Rafael Fernández-Leiro

CNIO- Spanish National Cancer Research Center

Telomeres are essential nucleoprotein structures located at the ends of chromosomes, evolved to protect and maintain genome integrity. The shelterin complex, a multiprotein assembly comprising six proteins - TRF1, TRF2, RAP1, TIN2, TPP1, and POT1 -, is located at telomeres and is responsible for their protection and length regulation. Misregulation of this complex is linked to numerous human diseases, including premature aging syndromes, telomeraseassociated
disorders, and cancer. Despite its importance, structural and mechanistic information regarding human shelterin components remains limited.
We adopted a multidisciplinary approach, using biochemistry, cryo-EM, and other biophysical and computational techniques to investigate how the shelterin complex is organised to safeguard chromosome ends and ensure proper telomere replication. After extensive optimisation efforts to generate all the components of this complex, we successfully isolated the individual shelterin proteins and assembled them into complexes. These reagents were used as tools to extract as much information as possible. Although atomic resolution structures remained elusive, likely due to the intrinsic flexibility of these proteins and their complexes, we employed other highly informative techniques for dynamic complexes, such as HDX-MS. Furthermore, we have incorporated AI tools into our research, focusing on identifying new protein-protein interactions. In particular, we have applied an in silico proteomics approach to identify new potential TRF1 interactors and discovered intriguing structural features that may serve as regulatory hubs. These findings not only enhance our understanding of the structural and functional dynamics of the shelterin complex but also open new avenues for therapeutic intervention, with potential applications in combating telomere-related diseases and cancer.

12 OCT4-SOX2 regulate BAF chromatin remodeling

Simone Cavadini[1], Luca Vecchia[1], Joscha Weiss[1,2], David Domjan[1,2], Georg Kempf[1], Lukas Kater[1], Nicolas H. Thomä[1,2]

1. Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland
2. EPFL, School of Life Sciences, Lausanne, Switzerland

How chromatin remodelers and transcription factors crosstalk structurally and functionally is unclear. Here we describe cryo-EM structures of the human TFs OCT4-SOX2 on nucleosomes together with the human BAF chromatin remodeler. We identify two majors binding modes, one where OCT4-SOX2 and BAF are spaced apart and do not interact; and another where SOX2 is wedged between the ATPase and the nucleosome with structural rearrangements of the ATPase, ARP and BASE modules. These structures provide an initial framework for rationalizing the finely tuned enzymatic responses to nucleosome-bound transcription factors *in vivo*.

13

Characterization of the dimeric yeast Cdc13 protein

Javier Coloma[1,*], Nayim Gonzalez-Rodriguez[1,*], Jaime Alegrio Louro[1], Neal F. Lue2, Oscar Llorca[1].

* These authors contributed equally to this work.
1. Structural Biology Programme, Spanish National Cancer Research Centre (CNIO), Madrid, Spain.
2. Department of Microbiology and Immunology, W. R. Hearst Microbiology Research Center, Weill Cornell Medicine, New York, United States.

The CST complex is involved in telomere replication, DNA repair, and chromosome cohesion. Its best-characterized functions are telomere capping and the regulation of telomere replication. The complex specifically binds to telomeric single-stranded DNA (ssDNA) regions in a sequence-specific manner. Additionally, it directly interacts with both telomerase and DNA polymerase alpha (Polα). These direct interactions—telomerase, which elongates the G-strand of telomeres, and Polα, which is responsible for the fill-in of the C-strand—place CST components at the center of telomere replication regulation. The complex consists of a large subunit—CTC1 in humans or Cdc13 in yeast—and two smaller subunits, STN1 and TEN1, which are well conserved across species.

Several structural studies of the human CST complex have revealed a decameric configuration when bound to DNA, and a heterotrimeric CTC1-STN1-TEN1 structure containing one CTC1 subunit when bound to Polα. However, Cdc13 does not resemble its mammalian counterpart CTC1, neither in sequence nor in domain organization. All yeast species analyzed to date show that Cdc13 dimerizes, but whether this dimerization mechanism is conserved across different yeast species remains unclear.

Our work with the CST complex from Candida glabrata has provided new insights into Cdc13 dimerization upon interaction with telomeric DNA. Our proposed model identifies the OB2 domain of CgCdc13 as the primary driver of protein dimerization. This contrasts with the model proposed for ScCdc13, where the OB1 domain is essential for dimerization. Our current efforts to validate this model are focused on the structural analysis of the Cdc13 dimerization core using both cryo-electron microscopy (cryo-EM) and structure prediction algorithms. To verify the structural information, point mutations in the predicted interaction interfaces are being analyzed using biophysical techniques.

14

Distinct Architectures of Polycomb Repressive Complex 2 Subtypes

Trinity Cookis, Alfredo Florez, Ana Galic, Alexandria Lydecker, Eva Nogales

University of California, Berkeley
Howard Hughes Medical Institute

Polycomb repressive complex 2 (PRC2) is an epigenetic regulator responsible for the trimethylation of histone H3 lysine 27, the chromatin mark associated with gene silencing. PRC2 function is in part regulated through the ability of the core complex to interact with diverse protein cofactors. The two major subtypes of PRC2 complexes, PRC2.1 and PRC2.2, regulate largely overlapping but distinct sets of genes and exhibit different catalytic and chromatin binding properties. The finetuned balance of PRC2.1 and PRC2.2 activities is essential for organism development and maintenance of cell identity. We present cryo-EM structures of diverse PRC2 complexes engaged with nucleosome substrates. We find that diverse PRC2 complexes engage catalytic substrates through conserved mechanisms, however, PRC2.1 and PRC2.2 complexes have distinct architectures and chromatininteracting surfaces that may influence targeting of PRC2 to chromatin. Our work further highlights the essential role of the PRC2 core subunit SUZ12 as a structural platform for PRC2 cofactor interactions and further supports that the catalytic activities of PRC2 may be decoupled from its recruitment to chromatin.

15

The in situ molecular architecture of chromatin in human cells revealed by high confidence 3D template matching

Sergio Cruz-León[1], Jan Philipp Kreysing[2], Johannes Betz[1], Carlotta Penzo[3], Tomáš Majtner[2], Markus Schreiber[2], Beata Turoňová[2], Marina Lusic[4], Gerhard Hummer[1], Martin Beck[2]

1. Department of Theoretical Biophysics, Max Planck Institute of Biophysics, Maxvon- Laue-Straße 3, 60438 Frankfurt am Main, Germany.
2. Department of Molecular Sociology, Max Planck Institute of Biophysics, Max-von- Laue-Straße 3, 60438 Frankfurt am Main, Germany.
3. Department of Infectious Diseases, Virology, Heidelberg University, Heidelberg, Germany.

Eukaryotic cells pack meters of DNA into micrometer-sized nuclei, creating a complex 3D chromatin structure that links molecular interactions to large-scale assemblies. Nucleosomes are the basic units of chromatin organization and have been extensively studied *in vitro*. However, their spatial arrangement within living cells is still largely unknown. Here, we use cryo-electron tomography (CryoET), our recently developed high-confidence 3D template matching (hcTM) method [1], subtomogram averaging, and molecular dynamics (MD) simulations to investigate chromatin organization at the nuclear periphery of primary human T cells. Using hcTM, we accurately identify the positions and orientations of individual nucleosomes in situ. We determine nucleosome connectivity by following the nucleosome-DNA linkers using a physics-based algorithm. The connected chromatin fragments provide us with a close-up view of the 3D genome organization along kilobase stretches of DNA, revealing an elongated chromatin architecture with a width of about 37 nm. We also investigate the dynamics of chromatin fragments by integrating CryoET and atomistic MD simulations. Nucleosome localization in situ with CryoET and hcTM thus paves the way for the mapping and simulation of nuclear landscapes in their native environment [2].

[1] Cruz-León, et al., Nat. Comm., 2024
[2] Kreysing*, Cruz-León*, Betz*, et al., BioRxiv, 2025

16

Trapping an Off-pathway Intermediate Enables Selective Chemical Inhibition of the RNA Helicase DDX19

Sourav Roy, Katelyn Baumer, Abigail Fish, Haley Marino, Judith Quadrozzi Gruntz, Jamie A. Jarusiewicz, Jake Slavish, Brandon Lee, Royappa Grace, Darcie Miller, and **Tommaso Cupido**

Department of Chemical biology and Therapeutics, St. Jude Children's Research Hospital

RNA helicases use ATP-driven conformational changes to remodel functional RNA structures and RNA-protein complexes (RNPs) essential for numerous RNA-based processes in normal and disease settings, including every step of the central dogma. Chemical inhibitors would be powerful tools to probe RNA helicase function and therapeutic potential, but structural conservation and conformational heterogeneity makes these enzymes highly challenging targets. Here we sought to delineate a strategy to harness the complex conformational dynamics of DEADBox ATPases (DDX), the largest class of RNA helicases, to develop selective inhibitors. We focused on DDX19, an enzyme that remodels the messenger RNP particle during transcription, nuclear export and translation. To define specific states within the DDX19 conformational ensemble that can be selectively modulated using small molecules, we used an approach that combines pre-steadystate kinetics, X-ray crystallography, and protein NMR. We show that the DDX19 core motor domain exists in slow (multi-second) equilibrium between an open and closed state. By high-throughput screening, we identified a compound that binds the open from's ATP pocket via conformational selection, followed by an inducedfit transition that stabilizes an inactive state, blocking ATPase and RNA-binding activities. Leveraging this induced-fit mechanism we developed SJ9877, a potent and selective DDX19 inhibitor. Structural analyses reveal that the induced-fit conformation represents an off-pathway state, rather than an ATPase cycle intermediate. Thus, SJ9877 showcases inactive-state trapping as a strategy for the chemical inhibition of DEAD-box ATPases. Inactive conformational states that lie outside the mechanochemical cycle provide a substrate for the evolution of new functions and regulatory mechanisms in molecular motors. Our findings suggest that these conformations are also attractive target for developing chemical probe through conformational trapping.

Other Information:
DEAD-Box ATPases are universal RNA-dependent molecular motors with essential functions in all phyla. In addition to their roles in basic RNA biology, these enzymes are highly valued as potential therapeutic targets, but we lack an understanding of their druggability. Our work provides structural and mechanistic insights into how selective inhibitors of DEAD-Box ATPases can be rationally developed. Our findings may be of broad interest to structural biologists that seek to target nucleic aciddependent motor proteins using small molecules.

17

Replication Under Pressure: Navigating DNA Repeats

Manuel Daza Martin and Gideon Coster

Instituto de Parasitología y Biomedicina

Accurate chromosomal replication is a fundamental requirement for genome stability. One major source of replication errors is repetitive DNA, which comprises over half of the human genome. DNA repeats can form unusual DNA secondary structures, such as hairpins, cruciforms, triplexes, G4 quadruplexes and i-motifs. Interestingly, expansion of repetitive sequences, mainly trinucleotide repeats, causes a wide range of neurodegenerative hereditary disorders and genetic instability. Therefore, it is crucial to uncover the pathways that ensure accurate replication of these structure-prone sequences. Here, we reconstituted replication of mono-, di- and trinucleotide repeats *in vitro* using eukaryotic replisomes assembled from purified proteins. We found that structure-prone repeats are sufficient to impair replication. Whilst template unwinding is unaffected, leading strand synthesis is inhibited, leading to fork uncoupling. Synthesis through hairpinforming repeats is rescued by replisome-intrinsic mechanisms, whereas synthesis of quadruplex-forming repeats requires an extrinsic accessory helicase. Altogether, the data presented here provides an exciting glimpse into how the replisome behaves as it encounters structured DNA and sets the grounds for better understanding of repeat expansion disorders.

18

The Role of Ku Threading in APLF-Mediated DNA End Bridging

Sara De Bragança[1], Raman van Wee[2,3], Eva M. Martin-Cuevas[1], Ángel Rivera- Calzada[4], Laia Romeu[1], Mikel Marín-Baquero[1], Raquel Arribas-Bosacoma[5], Clara Aicart-Ramos[1], Seham Helmi[1,2], Oscar Llorca[4], Philipp Kukura[2,3] and Fernando Moreno-Herrero[1]

1. Department of Macromolecular Structures, Spanish National Center for Biotechnology (CNB), Spanish National Research Council (CSIC), Madrid, Spain
2. Department of Chemistry, University of Oxford, Physical and Theoretical Chemistry Laboratory, OX1 3QZ, United Kingdom
3. Kavli Institute for Nanoscience Discovery, Biochemistry Building, OX1 3QU, United Kingdom
4. Structural Biology Programme, Spanish National Cancer Research Center (CNIO), Madrid, Spain
5. Genome Damage and Stability Centre, School of Life Sciences, University of Sussex, Brighton, UK

Ku and APLF are repair factors involved in the non-homologous end joining (NHEJ) pathway. In previous work, we demonstrated that Ku and APLF alone are capable of bringing the two ends of a double-strand break together; however, the mechanism underlying this structural role in DNA repair remains unclear. In a recent collaborative project, we dissected the Ku–APLF synaptic complex using atomic force microscopy (AFM), mass photometry (MP), and magnetic tweezers (MT). AFM imaging of 441 bp dsDNA fragments revealed that multiple Ku molecules can thread onto DNA, and the subsequent addition of APLF induces the formation of extensive macromolecular protein networks. Notably, MP results indicate that these networks form only when Ku is threaded onto the DNA, suggesting that the threading of Ku may generate a unique interface to which APLF binds. Additional experiments demonstrated that APLF requires two Ku molecules on one end for a successful DNA-end bridge, supporting our hypothesis that the generation of an interface on threaded Ku serves as the second APLF-binding region necessary to bridge the DNA ends. MT assays, using DNA substrates of varying lengths, further characterized the stability and kinetics of these complexes under applied force, showing that the number of threaded Ku molecules—and, consequently, the number of APLF molecules acting simultaneously—dictates the lifetime and strength of the DNA bridge. Truncation of the acidic C-terminal domain of APLF reveals that it plays a pivotal role in DNA synapsis by interacting with the Ku–Ku interface formed upon Ku threading, thereby facilitating DNA-end linkage. These findings provide novel insights into the architecture of Ku–APLFmediated synapsis, thereby providing a potential regulatory mechanism for how APLF-mediated bridging is restricted to Ku when it is bound to damaged DNA, and refine our understanding of the early steps in NHEJ, with potential implications for targeting genomic instability in disease.

19

Molecular Insights into Chloroplast Transcription Activation by Stress

Ashley P. Dudey, Michael Webster

John Innes Centre

Chloroplast transcription is central to the expression of proteins that perform photosynthesis. Like bacteria, chloroplasts rely on sigma factors for promoter recognition during translation initiation. Plant chloroplasts contain multiple sigma factors, each with specific promoter preferences, that facilitate the binding of the plastid-encoded RNA polymerase (PEP).
When plants encounter environmental stresses, Sigma factor 5 (SIG5) supports transcription from the blue light-responsive promoter (BLRP) upstream of the chloroplast psbD gene. This regulation is believed to support replacement of damaged D2 protein, a component of the photosystem II (PSII) reaction centre. Unlike canonical bacterial promoters, the BLRP lacks the -35 sequence element, and instead contains a conserved AAG motif upstream. A DNA-binding protein has been shown to interact with the AAG motif but remains unidentified.
We recently determined the first structural model of PEP [1]. To understand stress-responsive transcriptional regulation in plant chloroplasts, we are in the process of reconstituting the PEP-SIG5-BLRP transcription initiation complex for analysis by cryogenic electron microscopy (cryoEM). Here, I will summarize our recent mechanistic understanding of chloroplast transcription. I present our progress towards characterizing the molecular basis of chloroplast transcription initiation under stress, which could offer new strategies to enhance plant resilience to environmental challenges.

[1] Vergara-Cruces et al. (2024) Structure of the plant plastid-encoded RNA polymerase. Cell. 187(5):1145-1159. PMID: 38428394.

20

Insights into the R2SP quaternary molecular chaperone

Paulo E. Santo[1], Marie-Eve Chagot[2], Marie Ley[3,4], Hugo Gizardin-Fredon[3], Thomas Chenuel[5], Evolène Deslignière[3,4], Laura Plassart[1], Ana C. F. Paiva[6], Pedro M. F. Sousa[6], Edouard Bertrand[7], Bruno Charpentier[2], Céline Verheggen[7], Marc Quinternet[2], Philippe Meyer[5], Tiago M. Bandeiras[6], Sarah Cianférani[3,4,*], Célia Plisson-Chastang[1,*], Xavier Manival[2,*]

1. Molecular, Cellular and Developmental Biology unit (MCD), Centre de Biologie Integrative (CBI), University of Toulouse, France.
2. Université de Lorraine, IMoPA, Nancy, France
3. Laboratoire de Spectrométrie de Masse BioOrganique, Université de Strasbourg, Strasbourg, France
4. Infrastructure Nationale de Protéomique, Strasbourg, France
5. Sorbonne Université, Institut de Biologie Physico-Chimique, Laboratoire de Biologie Moléculaire et Cellulaire des Eucaryotes, Paris, France
6. iBET, Instituto de Biologia Experimental e Tecnológica, Oeiras, Portugal
7. IGH, CNRS, Univ Montpellier, France

The human R2SP complex belongs to the R2TP-like quaternary chaperone family and consists of RUVBL1, RUVBL2, SPAG1 and PIH1D2. R2SP is crucial for the correct assembly of motile cilia and the organization of the synaptic zone [1]. RUVBL1/2 ATPases are the powerhouse of this molecular machine, while SPAG1 and PIH1D2 would be adaptors that interact with specific clients to promote their quaternary assembly, assisted by Hsp70/90 cochaperones. Despite these functional data, little is known about the structure of R2SP and the precise mode of action of these R2TP-like complexes. We have combined biochemical and structural approaches (NMR, cryo-EM and structural mass spectrometry assays) to investigate the 3D organization of the human R2SP complex, its mode of assembly and ATPase activity [2]. Our study reveals a three-dimensional structure similar to that of the canonical R2TP complex, but also highlights differences in the mode of action of its RUVBL1/2 ATPase core as well as the binding of its adaptors SPAG1 and PIH1D2.

[1] - Maurizy, C., et al Nat Comms 9(1) (2018): 2093
[2] - Santo, P. E., et al bioRxiv (2025): 2025-01

21

Structural Basis for Human MRE11-RAD50-NBS1 bound to DNA and its Regulation by Telomeric Factor TRF2

Yilan Fan[1,3], Filiz Kuybu[1,3], Hengjun Cui[1,3], Katja Lammens[1], Jia-Xuan Chen[2], Michael Kugler[1], Christophe Jung[1], Karl-Peter Hopfner[1,4]

1. Gene Center, Department of Biochemistry, Ludwig-Maximilians-Universität München, Feodor Lynen Straße 25, 81377 Munich, Germany
2. Proteomics Core Facility, Institute of Molecular Biology, Mainz, Germany
3. These authors contributed equally
4. Lead contact

The MRE11-RAD50-NBS1 (MRN) complex is a central, multifunctional factor in the detection, signaling and nucleolytic processing of DNA double-strand breaks (DSBs). To clarify how human MRN binds generic and telomeric DNA ends and can separate DNA end sensing from nuclease activities, we determined cryo-electron microscopy structures of human MRN bound to DNA and to DNA and the telomere protection factor TRF2. MRN senses DSBs through a tight clamp-like sensing state with closed coiled-coil domains, but auto-inhibited MRE11 nuclease. NBS1 wraps around the MRE11 dimer, with NBS1's ATM recruitment motif sequestered by binding to the regulatory RAD50 S site, necessitating an allosteric switch for ATM activation. At telomeric DNA, TRF2 blocks the second S site via the iDDR motif to prevent nuclease and ATM activation. Our results provide a structural framework for topological DNA sensing and separation of sensing, signaling and processing activities of mammalian MRN.

22

Regulating R2TP function through artificial binder design

Nayim González Rodríguez[1], Carlos Chacón[1], Andrés López Perrote[1], Ana Isabel Hernández[1], Rafael Fernández Leiro[1,*], Óscar Llorca[1,*]

1. Spanish National Cancer Research Center (CNIO)
*. Implies equal contribution

Macromolecular interactions are fundamental to regulating cellular function and disease. Recent advances in diffusion algorithms enable the rational design of artificial proteins capable of regulating cellular function through specific proteinprotein interactions. We have developed a pipeline tailored for small GPU clusters, allowing parallel generation of thousands of designs from scratch in mini-batches of arbitrarily short jobs. Using this pipeline, we designed protein binders to inhibit the assembly of the R2TP complex, a complex responsible for assembling large macrocomplexes such as mTORC1, ATR, ATM, and DNA-PK. Specifically, we targeted the RUVBL1/2 hexamer, the core of R2TP, designing binders that occlude the RUVBL1-RPAP3 interaction site and thereby disrupt R2TP function. Our designs successfully bind the RPAP3 site and actively displace it from preassembled RUVBL1-RPAP3 complexes. Finally, we determined the high-resolution cryo-EM structure of RUVBL1 bound to one of our designed proteins, revealing full agreement between the computational model and the produced binder. These results show the power of our pipeline for generating targeted protein binders and provide a new strategy for modulating the R2TP complex function.

23

Spatial organization of mRNA with Ribosomes as single polymer chains and RNA structure predictions through multiscale and hierarchical methods

Horacio V. Guzman

1 Biophysics & Materials Eng. Group, Institut de Ciència de Materials de Barcelona, CSIC, 08193 Barcelona, Spain
2 Departamento de Física Teórica de la Materia Condensada, Universidad Autónoma de Madrid, 28049 Madrid, Spain

The assumed conformation of mRNA inside cells is formed based on the closedloop structure and internal base pairings with the activity of the ribosome movements. However, recent smFISH measurements [1] show that the closed-loop structure is not formed in many mRNAs. This implies that mRNA can be considered as a single polymer chain in the cell. Here, we introduce a model based on single-chain polymers and spherical-shaped Ribosomal segments, angular restraints between nucleotides (beads) [1] are applied to the mRNA segments that are not directly interacting with the ribosomes. Using Kremer-Grest parameters [2] and was able to recapitulate the mRNA conformation change of the translation activity and three-dimensional positional interaction between the organelle and its localized mRNAs as end-to-end (E2E) distances. Remarkably, the coefficient of the scaling law suggests that the presence of ribosomes increases the blob-size, which at the same time reduces the repulsive interactions between blobs and hence increases the E2E distance of those mRNAs. Our method is also used to tackle fundamental questions like the nature of the interactions between ribosomes, and the origin of their spatial configurations inside the cell. We finally also discuss the interpretation of the aggregation mechanism of mRNA under different cellular conditions such as translation activity, based on the insights from our model.
In the second part of this poster I will present a structural prediction pipeline [3,4], based on building 3D coarse-grained structures at nucleotide level resolution and then refining them towards all-atom representations.

[1] T. Guo, O. L. Modi, J. Hirano, H. V. Guzman, T. Tsuboi, Biophysical Reports 2022, 2 (3), 100065.
[2] H. Kobayashi, H. V. Guzman, In submission, 2025
[3] Bu, F, et al., RNA-Puzzles Round V: Blind predictions of 23 RNA structures, Nature Methods, https://doi.org/10.1038/s41592-024-02543-9 (2025).
[4] https://tinyurl.com/goCompModeling

24

Single-Molecule Insights into Human Mitochondrial DNA Helicase Autoregulation

Ismael Plaza-G.A., Seth P. Buchanan, Samuel Míguez, Rafael Fernandez-Leiro, Grzegorz L. Ciesielski, **Borja Ibarra**

Instituto Madrileño de Estudios Avanzados en Nanociencia, IMDEA Nanociencia, Spain.
University of North Florida, Jacksonville, FL, United States.
Spanish National Cancer Research Centre (CNIO), Madrid, Spain.

The human mitochondrial helicase Twinkle is essential for mitochondrial DNA (mtDNA) replication and integrity. Using biochemical and single-molecule techniques, we investigated Twinkle's real-time kinetics, including DNA loading, unwinding, and rewinding, and their regulation by its N-terminal Zinc-binding domain (ZBD), C-terminal tail, and mitochondrial SSB protein (mtSSB). Our results indicate that Twinkle rapidly scans dsDNA to locate the fork, where specific interactions halt diffusion. During unwinding, ZBD-DNA interactions and Cterminal control of ATPase activity downregulate kinetics, slowing down the helicase. Binding of mtSSB to DNA likely outcompetes ZBD-DNA interactions, alleviating the down regulatory effects of this domain. Furthermore, we show that ZBD-DNA interactions and ATP binding also regulate rewinding kinetics following helicase stalling. Our findings reveal that ZBD and C-terminal tail play a major role in regulation of Twinkle´s real-time kinetics. Their interplay constitutes an autoregulatory mechanism that may be relevant for coordinating the mtDNA maintenance activities of the helicase.

25 From Open to Active: Structural Basis for sRNA Cleavage by a Conserved Bacterial Ribonuclease

Kai Katsuya-Gaviria, Aleksei Lulla, Giulia Paris, Ben Luisi

Department of Biochemistry, University of Cambridge

Bacterial small RNAs (sRNAs) are central to post-transcriptional gene regulation, especially under stress. In Escherichia coli, RyhB is a well-established model for sRNA-mediated regulation that modulates iron homeostasis by controlling the stability and translation of over 20 mRNA targets via base-pairing. While RyhB's regulatory roles are well studied, the mechanisms controlling its degradation remain unclear. Here, we characterize the cleavage of RyhB by a widely conserved yet recently discovered ribonuclease, YicC, providing new insights into targeted sRNA turnover.

Using *in vitro* assays, we mapped a precise cleavage site within RyhB. Cryo-electron microscopy revealed that the ribonuclease exists in an inactive, open conformation in the apo state. Upon binding RyhB, the enzyme undergoes a large structural rearrangement into a catalytically competent, closed conformation. This RNAinduced structural switch repositions catalytic elements, enabling highly selective cleavage.

To investigate its role *in vivo*, we are using northern blotting to validate the cleavage site and assess its activity under iron-limiting conditions. In parallel, structure-guided mutagenesis is being used to probe key residues involved in the conformational switch. The functional consequences of these mutations are assessed using a fluorescent reporter assay, where an mRNA target of RyhB, sodB, is fused to a fluorescent protein coding sequence. Disrupting ribonuclease activity stabilizes RyhB, increasing sodB repression and reducing fluorescence, providing a live-cell readout of sRNA degradation.

Comparative sequence analyses reveal that this ribonuclease is widely conserved across Gram-positive and Gram-negative bacteria, including species that lack RyhB. This conservation suggests the enzyme targets additional, yet unidentified, RNA substrates, indicating a broader role in bacterial RNA regulation beyond iron metabolism.

26 The AAA-ATPase Rix7 and ribosome biogenesis: structural and functional insights into Mak16-mediated ATPase regulation

Natalia Kulminskaya, Alina Sophie Thielen, Matthias Thoms, Roland Beckmann, Valentin Mitterer

Ribosome biogenesis is a highly complex and energyintensive process, involving coordinated assembly and remodeling events across nuclear compartments. The AAA+ ATPase Rix7 plays a critical role in this pathway by mediating the release of assembly factors, including the Nsa1-module, to promote 60S subunit maturation. In this study, we investigate the structural and functional interactions between Rix7 and Mak16, a core component of the Nsa1-module. Among its module partners, Mak16 exhibits the strongest affinity for Rix7 and emerges as its primary binding partner. We found that the dominant-negative Rix7 E634Q mutant enhances Mak16 binding and causes mislocalization of Nsa1-module components, and we further explore this interaction using truncation mutants. Our data reveal that the E634Q mutation and C-terminal truncation/deletion of Mak16 significantly affect binding affinity and alter the stoichiometry of the Rix7–Mak16 complex. These findings are supported by AlphaFold modeling, which highlights a broad interaction interface. Optimization of protein purification and assay conditions demonstrated that an additional SEC polishing step is critical for robust ATPase activity, with Mak16 directly enhancing Rix7's hydrolysis capacity. While biochemical characterization is ongoing, our results provide new mechanistic insight into Rix7 function and lay the groundwork for dissecting cofactor-specific regulation during ribosome biogenesis.

cnio - CaixaResearch
FRONTIERS
MEETINGS

Madrid 28th – 30th May 2025

Machines acting on DNA and RNA, a molecular mechanistic perspective

Thursday May 29th 2025

Poster Session group 2

cnio stop cancer

27

Structural basis of multitasking by the apicoplast DNA polymerase from Plasmodium falciparum.

Anamika Kumari[1], Theodora Enache[1], Janice Pata[2,3], Indrajit Lahiri[1,4,5]

1. School of Biosciences, Molecular Microbiology, The University of Sheffield, Sheffield, UK
2. New York State Department of Health, Wadsworth Center, Albany, New York, USA
3. State University of New York at Albany, Abany, New York, USA
4. Nucleic Acids Institute, The University of Sheffield, Sheffield, UK
5. Florey Institute of Infection, The University of Sheffield, Sheffield, UK

Plasmodium, the causative agent of malaria, harbours a unique, essential, nonphotosynthetic plastid called the apicoplast. Apicoplast maintains its own genome (apDNA), duplication of which is essential for Plasmodium survival. apDNA duplication is performed by a dedicated replisome containing a minimal set of proteins. Long term, we want to understand how the stripped-down Plasmodium apicoplast replisome faithfully copies apDNA within the hostile environment inside the human host. As a first step, I have started working on the sole apicoplast DNA polymerase (apPol), the core component of the apicoplast replisome. Being the only DNA polymerase present in the apicoplast, apPol has the unique ability to perform replicative synthesis as well as lesion bypass synthesis, roles that are performed by distinct, specialized DNA polymerases in cellular replisomes. Using single particle electron cryomicroscopy, we have solved the structure of apPol bound to its DNA and nucleotide substrates in multiple conformational states as the enzyme prepares to add a nucleotide to the nascent DNA strand. I will discuss our analysis of these structures and explain how apPol balances replicative and lesion bypass synthesis.

28

Structure and force-activation of focal adhesion signaling

Karen Diaz Palacios, Pilar López Navajas, Bárbara Rodrigo Martín, Ruth Matesanz, Juan R. Luque-Ortega, Asier Echarri and **Daniel Lietha**

Margarita Salas Center for Biological Research (CIB), Spanish National Research Council (CSIC), Madrid, Spain.

The Focal Adhesion (FA) complex plays a key role in mesenchymal cell migration and during tumor progression enables cancer cells to invade neighboring tissues and initiate metastasis. FAs act as the “feet” of the cell in that they attach to the extracellular matrix (ECM) outside the cell and build-up traction by connecting to actin stress fibers inside the cell. The force generated by contracting stress fibers is transmitted across FAs via force transduction proteins. These proteins on the one hand link actin to ECM bound integrin receptors for traction, and on the other hand connect to a membrane bound signaling layer in FAs. The latter allows for force induced activation of FA signaling, which is critical for coordinated FA maturation and disassembly at the cell rear to ensure processive migration. A long standing question in the field is how the mechanical force in FAs is converted into a biochemical signal.

A key signaling molecule in FAs is Focal Adhesion Kinase (FAK), which is activated with increasing forces in FAs. A first clue how force might activate FAK came from our cryo-EM structure which shows that FAK initially integrates into FAs as an oligomer that is tightly binds to the membrane and in which FAK adopts an inactive conformation where the kinase active site is occluded by the membrane. With the FAK C-terminus known to interact with FA force transduction proteins, this immediately suggested that force transmitted via the FAK C-terminus could lift the kinase off the membrane for activation.

Here we present data, including new structural information, that supports such a mechanism and suggests that force is transmitted to FAK via the force transduction protein vinculin and a paxillin tether that links vinculin to the FAK C-terminus. We further show that the linkage to vinculin is regulated and requires phosphorylation of the FAK C-terminus. In summary, we provide an atomic model for phosphoregulated force-activation of FA signaling.

29

Hydrolytic endonucleolytic ribozyme (HYER) is programmable for sequencespecific DNA cleavage

Zi-Xian Liu†, Shouyue Zhang†, Han-Zhou Zhu†, Zhi-Hang Chen†, Yun Yang†, Long- Qi Li†, Yuan Lei, Yun Liu, Dan-Yuan Li, Ao Sun, Cheng-Ping Li, Shun-Qing Tan, Gao-Li Wang, Jie-Yi Shen, Shuai Jin, Caixia Gao, Jun-Jie Gogo Liu*

Beijing Advanced Innovation Center for Structural Biology, State Key Laboratory of Membrane Biology, Tsinghua-Peking Center for Life Sciences, School of Life Sciences, Tsinghua University, Beijing 100084, China.
New Cornerstone Science Laboratory, Center for Genome Editing, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing, China.
College of Advanced Agricultural Sciences, University of Chinese Academy of Sciences, Beijing, China

Ribozymes are catalytic RNAs with diverse functions including self-splicing and polymerization. This work aims to discover natural ribozymes that behave as hydrolytic and sequence-specific DNA endonucleases, which could be repurposed as DNA manipulation tools. Focused on bacterial group II-C introns, we found that many systems without intron-encoded protein propagate multiple copies in their resident genomes. These introns, named HYdrolytic Endonucleolytic Ribozymes (HYERs), cleaved RNA, single-stranded DNA, bubbled double-stranded DNA (dsDNA), and plasmids *in vitro*. HYER1 generated dsDNA breaks in the mammalian genome. Cryo–electron microscopy analysis revealed a homodimer structure for HYER1, where each monomer contains a Mg2+-dependent hydrolysis pocket and captures DNA complementary to the target recognition site (TRS). Rational designs including TRS extension, recruiting sequence insertion, and heterodimerization yielded engineered HYERs showing improved specificity and flexibility for DNA manipulation.

30

Understanding the HSP90-mediated assembly and maturation of the PIKK complexes.

Alvaro L. Codina, Carmen García-Martín, Ana González-Corpas, Maria Martinez- Molledo, Andrés López-Perrote, Maria I. Dauden, Oscar Llorca*.
*Corresponding author.

Spanish National Cancer Research Centre (CNIO), Structural Biology Programme, Madrid, Spain.

PIKKs (phosphatidylinositol 3-kinase-related kinases) are a family of serinethreonine kinases that regulate DNA and RNA metabolism and are active as part of larger multi-subunit complexes [1]. In detail, mTOR (mammalian target of rapamycin) forms the mTOR complex 1 (mTORC1) and complex 2 (mTORC2) to regulate cell growth [2], SMG1 (suppressor of morphogenesis in genitalia 1) participates in the initiation of nonsense-mediated mRNA decay in complex with SMG8 and SMG9 [3], and ATR (ataxia- and Rad3-related) forms a complex with ATRIP (ATR-interacting protein) involved in the DNA damage response [4]. The assembly of these PIKK complexes is a multi-step process requiring the HSP90 (heat shock protein 90) chaperone working in concert with the TELO2–TTI1–TTI2 (TTT) and the RUVBL1–RUVBL2–RPAP3–PIH1D1 (R2TP) co-chaperone complexes [5],[6]. However, the molecular mechanism and regulation of the PIKK assembly pathway remain obscure due to the lack of structural information.
In our group, we are interested in the molecular bases for the assembly and activation of the mTORC1 and SMG1-SMG8-SMG9 complexes. First, we are aiming to elucidate how HSP90 stabilizes mTOR and SMG1 to assemble mTORC1 and SMG1-SMG8-SMG9, respectively. For this, we are optimizing the recombinant expression and purification of HSP90, mTOR and SMG1 in insect cells, aiming to obtain HSP90–mTOR and HSP90–SMG1 complexes by both *in vitro* reconstitution and co-expression. Structural analyses of the purified complexes will then be performed by cryo-EM. We expect that the comparison between the intermediate assembly states of both mTORC1 and SMG1-SMG8-SMG9 will help us to uncover conserved and specific features of the mechanism employed by the HSP90–R2TP–TTT system for the assembly and maturation of PIKK complexes.

Other Information:
In the abstract, numbers between brackets respectively correspond to the following references:
[1] Imseng S. et al. (2018), Curr. Opin. Struct. Biol. 49, 177-189
[2] Linde-Garelli K. Y. and Rogala K. B. (2023), Curr. Opin. Struct. Biol. 82, 102663
[3] Langer L. M. et al. (2020), eLife 9, e57127
[4] Rao Q. et al. (2018), Cell Res. 28, 143-156
[5] Toullec D. et al. (2021), Cell Rep. 37, 109867
[6] Dauden M. I. et al. (2021), Curr. Opin. Struct. Biol. 67, 78-85

31

Structure of CONCR, a lncRNA that regulates DNA replication and chromatid cohesion

Andrés López-Perrote[1*], Eva M. Martin-Cuevas[2*], José A. Mérida-Cerro[3*], Ana González-Corpas[1], Clara Aicart-Ramos[2], Johanne Le Coq[1], José M. Fernández- Justel[3], Jasminka Boskovic[1], Isabel Chillón[4$], Maite Huarte[3$], Fernando Moreno-Herrero[2$] and Oscar Llorca[1$]

1. Spanish National Cancer Research Centre (CNIO), Structural Biology Programme, Madrid, Spain.
2. Department of Macromolecular Structures, Centro Nacional de Biotecnología (CNB), CSIC, Madrid, Spain
3. RNA biology and Therapies Program. Center for Applied Medical Research (CIMA), University of Navarra, Pamplona, Spain.
4. Institute of Molecular Genetics of Montpellier (IGMM), CNRS, Montpellier, France.

*. Equal contributors.
$ Co-corresponding authors

The long-noncoding RNA (lncRNA) CONCR (cohesion regulator noncoding RNA) plays a crucial role in regulating DNA replication and sister chromatid cohesion by modulating the activity of DDX11 helicase (1). Remarkably, its expression is significantly elevated in various cancer types (1,2). Recent studies highlighting the structural-functional relationships of lncRNAs prompted us to investigate the structure of CONCR and its influence on functional mechanisms, an area that remains largely unexplored. We have combined biochemistry, SHAPE-MaP (Selective 2'-Hydroxyl Acylation analyzed by Primer Extension and Mutational Profiling), atomic force microscopy (AFM), and cryo-electron microscopy (cryo-EM) to elucidate the structure of CONCR. Our findings indicate that the lncRNA comprises multiple structural domains interconnected by flexible regions. Notably, the 3'-end of CONCR, specifically the segment encompassing nucleotides 419 through 718 (CONCR419-718), forms a structural domain that efficiently interacts with DDX11 *in vitro*. The preliminary three-dimensional structure of the CONCR419-718 domain, determined by cryo-EM, reveals a well-organized Lshaped architecture. Collectively, our results uncover a structurally defined domain within CONCR that is both necessary and sufficient for DDX11 binding. These findings support the idea that lncRNAs are organized into discrete structural domains with specific functional activities, interconnected within a larger flexible framework. Such organization may have important implications for understanding the regulatory roles of lncRNAs in cellular processes, particularly in cancer biology.

Other Information: References

(1) Marchese FP, Grossi E, Marín-Béjar O, Bharti SK, Raimondi I, González J, Martínez-Herrera DJ, Athie A, Amadoz A, Brosh RM Jr, Huarte M. 2016. A Long Noncoding RNA Regulates Sister Chromatid Cohesion. Mol Cell. 63(3):397-407.

(2) Calì F, Bharti SK, Di Perna R, Brosh RM Jr, Pisani FM. 2016. Tim/Timeless, a member of the replication fork protection complex, operates with the Warsaw breakage syndrome DNA helicase DDX11 in the same fork recovery pathway. Nucleic Acids Res. 44(2):705-17.

32

Deciphering the novel repression mechanism present in SPβ-Like Phages

Zamora-Caballero S, Chmielowska C, Gallego Del Sol F, Mancheño-Bonillo ., Eldar A, Penadés JR, **Marina A.**

-Instituto de Biomedicina de Valencia (IBV)-CSIC and CIBER de Enfermedades Raras (CIBERER)-ISCIII, Valencia, Spain.
-Centre for Bacterial Resistance Biology, Imperial College London, London, UK.
-School of Health Sciences, Universidad CEU Cardenal Herrera, CEU Universities, Alfara del Patriarca, Spain.
-Shmunis School of Biomedicine and Cancer Research, Faculty of Life Sciences, Tel- Aviv University, Tel-Aviv, Israel.

Temperate bacteriophages can alternate between virulent lytic and dormant lysogenic cycles. In the λ phage, this decision hinges on a bistable switch between two key regulators, CI and Cro, which control transcription of most phage genes. CI acts as the master repressor, binding cooperatively to cell-cycle operators to repress lytic genes and maintain lysogeny. Host DNA damage triggers the SOS response, activating RecA, leading to CI autoproteolysis and initiating the lytic cycle.

In contrast, SPbeta-like phages utilize the arbitrium communication system to regulate the lysis-lysogeny decision. This system employs a signaling peptide, AimP, produced during infection, which is secreted and taken up by neighboring bacteria. Inside the cell, AimP binds to the transcriptional regulator AimR, altering its activity. At low AimP concentrations, AimR activates AimX expression, promoting the lytic cycle. As AimP accumulates, it binds to AimR, inhibiting AimX expression and favoring lysogeny. This quorum-sensing-like mechanism allows the phage population to collectively decide between lytic and lysogenic pathways based on AimP concentration, reflecting the density of previous infections.

Our recent structural and functional studies on the phi3T phage reveal that its master repressor, SroF, differs significantly from those in λ-like phages. SroF adopts an integrase-like fold and binds DNA as a monomer, interacting with a linear and long DNA recognition motif. Furthermore, SroF-mediated repression is relieved not through proteolytic cleavage but via interaction with a third-party protein, phi3T_99. Notably, expression of phi3T_99 is regulated by an SOS box, directly linking the host SOS response to phage induction. Our findings elucidate the unique molecular mechanisms of SroF-phi3T-mediated repression, underscoring the variety of regulatory strategies employed by temperate phages to control their life cycles, adapting to different host environments and ecological niches.

33

Mechanisms of Mitochondrial DNA Replication: Structural characterization of the DNA Polymerase Gamma

Míguez-Amil, Samuel; Grande-García, Araceli, Fernández-Leiro, Rafael

Genome Integrity and Structural Biology Group, Structural Biology Programme,
Spanish National Cancer Research Centre, CNIO, Madrid, Spain

Mitochondrial metabolism is increasingly recognized as a key player in neurological and neurodegenerative diseases. Mutations in nuclear-encoded genes required for mitochondrial DNA (mtDNA) maintenance can cause severe neurological disorders. In parallel, structural defects in mtDNA have been linked to both neurodegeneration and cancer. The accurate replication of mtDNA by the mitochondrial replisome is essential for maintaining genome integrity and overall mitochondrial function. However, the molecular mechanisms governing this process remain incompletely understood. Our work aims to dissect the replication machinery, with a particular focus on DNA polymerase gamma, the main replicative enzyme in mitochondria. Here, we present recent structural and biochemical insights that shed light on its function and regulation. Understanding these mechanisms is fundamental to clarifying the molecular basis of mtDNA-related pathologies and may guide the development of novel therapeutic strategies.

34

A top-down approach for studying the molecular mechanisms of vertebrate DNA replication and repair by cryo-EM

Christl Gaubitz[1], Ioana Maruntel[1], Manil Kanade[1], Marvin Weiler[1], Xueyuan Leng[2,3], Ivo A. Hendriks[4], Alexandra Avram[1], Tillmann Pape[5], Nicholas Sofos[5], Ulrike Kühbacher[2,3], Michael L. Nielsen[4], Julien P. Duxin[2,3], **Thomas C.R. Miller[1]**

1. DNRF Center for Chromosome Stability, Department of Cellular and Molecular Medicine, University of Copenhagen, Denmark
2. NNF Center for Protein Research, University of Copenhagen, Copenhagen, Denmark
3. Biotech Research and Innovation Centre, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark
4. NNF Center for Protein Research, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark
5. Core Facility for Integrated Microscopy (CFIM), Faculty of Health and Medical Sciences, University of Copenhagen, Denmark

Faithful genome replication is essential for controlled cellular proliferation and the stable propagation of genetic material between generations. To ensure that complete genome replication occurs once-and-only-once per cell cycle, eukaryotes have evolved complex multi-step mechanisms to regulate precisely when and where the replication machinery is assembled and activated. To investigate these mechanisms, we have developed a 'top-down' approach to enrich DNA replication intermediates on synthetic DNA substrates from the near-native environment of Xenopus lævis egg extracts for imaging by electron microscopy. We have characterised conditions for efficient loading of replication-competent MCM helicases onto DNA, confirming that Xenopus MCM loading is stimulated by Orc6, requires Cdt1 and is inhibited by geminin. Moreover, we have determined a 2.7 Å cryo-EM structure of a DNA-bound Xenopus lævis MCM double hexamer (MCMDH), demonstrating the feasibility of obtaining high-resolution EM structures of replication intermediates purified from these extracts by DNA pulldown. Like human MCM-DHs (but unlike those in saccharomyces cerevisiae), Xenopus MCMDHs use highly conserved residues located in symmetrically opposed MCM5 zinc finger domains to untwist duplex DNA at the MCM dimer interface, disrupting a single base pair. This proof-of-principle study represents the first step towards the structural characterisation of vertebrate DNA replication reconstituted in Xenopus lævis egg extracts and offers the potential for determining the mechanisms and interplay between diverse molecular machines that act on DNA.

35

Towards long-term imaging of single mRNAs and their associated translation factors

Agata D. Misiaszek[1], Jeffrey A. Chao[1]

1. Friedrich Miescher Institute for Biomedical Research (FMI), Fabrikstrasse 24, 4056 Basel

Single-molecule imaging of mRNAs in live cells provides high spatial and temporal resolution, enabling the detailed study of molecular processes and mechanisms. Recent technological advances have facilitated investigations in fixed cells and short-term live-cell studies, revealing key aspects of RNA behavior. However, long-term measurements and studies of protein behavior in cells remain challenging, limiting the range of biologically relevant questions that can be addressed.
To enable long-term single-mRNA observations, transcripts must be immobilized. We employed a system in which transcripts are anchored at the plasma membrane via the CAAX motif. To tether the mRNA, we utilized the 4×ʎN22 peptide, which binds the BoxB RNA motif with high affinity. This immobilization allows extended imaging, enabling the quantification of key parameters such as ribosome occupancy, translation initiation rates, and kon/koff dynamics throughout the lifetime of an mRNA.
Live-cell single-molecule studies have primarily focused on the RNA, as signal amplification can be achieved using arrays of epitopes. However, single-protein imaging requires direct labeling with fluorophores that provide sufficient signal-to-background contrast. We tested multiple constructs and selected the "spaghetti monster" system, which incorporates 18 ALFA-tag repeats bound by a fluorescent nanobody. As a proof of principle, we fused this fluorescent construct to the PP7 bacteriophage coat protein (PCP), which stably associates with PP7 RNA loops. By varying the number of PP7 loops, we modulated the number of bound PCP molecules to assess detection limits. Having validated this approach, the selected tag can now be tested on biologically relevant proteins.
Imaging single proteins associated with immobilized mRNA expands the capabilities of single-molecule studies, enabling longer observation times and enhanced readouts. This approach can be applied to investigate translation factors and their influence on mRNA translation status. Furthermore, it provides a powerful tool to study how different transcripts are regulated under various conditions, such as cellular stress.

References
[1] Livingston NM, et al. (2022) Bursting translation on single mRNAs in live cells. bioRxiv, 10.1101/2022.11.07.515520.
[2] Daigle N, Ellenberg J (2007) LambdaN-GFP: an RNA reporter system for live-cell imaging. Nat. Methods., 4(8): 633-636.
[3] Götzke H, et al. (2019) The ALFA-tag is a highly versatile tool for nanobody-based bioscience applications. Nat. Comm., 10:4403.
[4] Chao JA, et al. (2007) Structural basis for the coevolution of a viral RNA-protein complex. Nat. Struct. Mol. Biol., 15:103-105.

36

A Ring to Regulate it all: a CRISPR story

Rafael Molina[1,2], Stefano Stella[2], Mingxia Feng[3,4], Nicholas Sofos[2], Vykintas Januiskis[2], Irina Pozdnyakova[5], Blanca Lopez-Mendez[2], Anne Louise Grøn Jensen[2], Javier Marchena-Hurtado[2], Ricardo Garcia-Martin[2], Qunxin She[3,4], and Guillermo Montoya[2].

1. Department of Crystallography and Structural Biology, Institute of Physical Chemistry Blas Cabrera, Spanish National Research Council, Madrid, (Spain).
2. Structural Molecular Biology Group, Novo Nordisk Foundation Centre for Protein Research, Faculty of Health and Medical Sciences University of Copenhagen (Denmark).
3. College of Life Science and Technology, Huazhong Agricultural University, Wuhan, (China).
4. Archaea Center, Department of Biology, University of Copenhagen, Ole Maaløes Vej 5, Copenhagen Biocenter, Copenhagen (Denmark).

CRISPR loci and CRISPR-associated (Cas) genes encode an adaptive immune system that protects many bacterial and almost all archaea against invasive genetic elements from bacteriophages and plasmids. Several classes of CRISPR systems have been characterized, of which the type III CRISPR systems exhibit the most unique functions. Members of type III cleave both RNA and DNA not only through their corresponding effector complexes but also by CRISPR-Cas associated proteins activated by second messengers produced by those effector complexes. Furthermore, the recent discovery of second messenger degrading proteins called ring nucleases adds an extra regulatory layer to fine-tune these immunity systems. Here, we present our findings about the defense mechanisms that govern type III CRISPR interference immunity systems, focusing on the structural information.

(1-5).
1. Molina, R*. et al. Molecular Basis of cyclic tetraoligoadenylate processing by small standalone CRISPR-Cas Ring Nucleases. Nucleic Acids Research 50, 19, 11199– 11213 (2022).
2. Molina, R*. et al. Structural basis of cyclic oligoadenylate degradation by ancillary Type III CRISPR-Cas Ring Nucleases. Nucleic Acids Research 49, 21, 12577-12590 (2021).
3. Molina, R. et al. Structural basis of CRISPR-Cas Type III prokaryotic defence systems. Current Opinion in Structural Biology, 65, 119-129 (2020).
4. Sofos, N. et al. Structures of the Cmr-beta complex reveal the regulation of the immunity mechanism of Type III-B CRISPR-Cas. Molecular Cell, 79, 741-757 (2020).
5. Molina, R. et al. Structure of Csx1-cOA4 complex reveals the basis of RNA decay in Type III-B CRISPR-Cas. Nature Communications, 10, 4302 (2019).

37

Molecular Mechanisms of Chloroplast Transcription Regulation

Jessica Moon

John Innes Centre, Biotechnology and Biological Sciences Research Council (BBSRC)

Photosynthesis is essential to growth and adaptation of plants to different environmental conditions. Expression of genes encoded in the chloroplast genome underpins photosynthesis, yet our understanding of how the transcription of these genes is regulated is limited.
Recently, we solved the first cryo-EM structure of the Plastid-Encoded RNA Polymerase (PEP), which showed it to be composed of a core similar to cyanobacterial RNAP surrounded by fifteen additional, nuclear-encoded factors termed PEP-Associated Proteins (PAPs). The chloroplast has lost most of the transcriptional regulators present in its cyanobacterial ancestor. It is therefore likely that nuclear-encoded factors both compensate for this loss and potentially perform functions unique to the chloroplast.
To develop our understanding of transcriptional regulation in the chloroplast, we aim to identify potential transcriptional regulators using experimental and computational approaches and investigate the mechanisms by which they regulate transcription by biochemical and structural methods. Here, I will summarise our work on the structure of PEP and how it has enhanced our understanding of transcription in the chloroplast. I will also present our progress in identifying and characterizing the mechanisms of chloroplast transcriptional regulators.
An understanding of transcriptional regulation in the chloroplast will provide a foundation for the development of plants with more robust photosynthetic gene expression.

38

The structure of the human DDX11 helicase

A. Longo, L. Koziej, M. Jaciuk, L. De Colibus L.M.R. Napolitano, S. Glatt and **S. Onesti**

Elettra-Sincrotrone Trieste (IT)
Małopolska Centre of Biotechnology, Jagiellonian University, Krakow (PL)

Helicases are essential and ubiquitous enzymes, playing a key role in a variety of processes in DNA and RNA metabolism. A subset of helicases play specialised and specific functions by resolving/remodelling a variety of atypical DNA structures, such as G-quadruplexes, triplexes, Holliday junctions, as well as displacement loops (D-loops and R-loops): among those a major role is played by the FeS family. Helicases containing FeS-clusters are ubiquitous but their exact mechanism of action is poorly understood; in particular, no structural information are available for some medically-relevant members of the family, like FANCJ, DDX11 and RTEL1.
The combination of the intrinsic conformational flexibility, FeS cluster lability and size makes them challenging targets for structural biology.
DDX11 plays an important role in sister-chromatid cohesion, associates with the replisome and is involved in processing non-canonical nucleic acid structures.
We have expressed and purified the human protein with an intact FeS cluster and carried out an extensive biochemical characterization, and we have demonstrated its ability to unwinding physiologically-relevant unimolecular G4. We have collected Cryo-EM data for the protein alone in complex with a DNA fork: the apo structure is currently being refined at 3.5Å resolution and a preliminary 5 Å structure in a complex with a DNA fork has been determined. We can clearly see the path of the DNA fork bound to the helicase including the double helix, and the 5' single strand across the motor domains. Interestingly, in same regions the structure differs significantly from the AlphaFold model. These structures provide an essential framework to better understand the role of these enzymes, to elucidate their mechanism of action in the processing of non-canonical nucleic acid structures, (such as G-quadruplexes, D-loops and R-loops), and to shed light on their involvement in genetic diseases and cancer development.

39

Single-molecule manipulation of genome integrity guardians

María Ortiz Rodríguez, Roberto Galletto and Borja Ibarra

Molecular Motors Manipulation Lab (IMDEA Nanociencia, Faraday 9, 28049, Madrid, Spain)
Department of Biochemistry and Molecular Biophysics (Washington University School of Medicine, St. Louis, Missouri, USA)

DNA helicases of the Pif1 family are essential for maintenance of nuclear and mitochondrial genomes, acting as a guardian of its integrity. In coordination with other protein partners, Pif1 helicases work as molecular motors that unwind duplex nucleic acids and remodel nucleic acid-protein complexes driven by the energy from ATP hydrolysis. How these motor proteins open the DNA, how they couple chemical (ATP binding and hydrolysis) and mechanical (DNA unwinding) reactions, and how their behavior is modulated by protein-partners, such as singlestranded
DNA binding (SSB) proteins, are questions under intense debate. To address these questions, we have developed an optical tweezers assay to follow in real-time and manipulate mechanically the DNA unwinding activity of individual Pfh1 helicase molecules. Here, we describe the effect of fork stability and ATP concentration on the real-time DNA unwinding kinetics of the Pfh1 helicase to unravel the mechano-chemical processes (energy conversion to work) that govern its operation. In addition, we show how binding of spRim1 SSB proteins to DNA modulates the real-time kinetics of the helicase.

Other Information:
Keywords: Helicase Pifh1, SSB spRim1, Optical Tweezers, DNA maintenance,
Supported by: Spanish Ministry of Science and Universities, PID2021-126755NB-I00

40

Structural studies of the Mediator complex and the Mediator kinase modules

Si Hoon Park[1], Lukas Kater[1], Georg Kempf[1], Alessandro Minafra[2], Alexander Hanzl[1], Alexandra Bendel[2], Manuel Carminati[2], Mira Schuetz[1], Gabriele D'Alessandro, Daniel Hess[1], Simone Cavadini[1], Nicolas H. Thomä[1,2]

1. Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland
2. School of Life Sciences, École Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland

Transcription regulation is essential for proliferation and differentiation in eukaryotic cells. In eukaryotes, transcription factors (TFs) recognize specific DNA binding motifs through their DNA binding domains (DBDs), while their effector domains (EDs) interact with Mediator complex or transcriptional coregulators (CRs) to activate or repress transcription. The Mediator complex, a key activator for transcription, has a molecular weight of 1.4 MDa and comprises 26 subunits. It is regulated by two different Mediator kinase modules, MKMCDK8 and MKMLCDK19, each consisting of four subunits including CyclinC, MED12/12L, and MED13/13L. Mediator and its kinase modules recognize numerous transcription factors at enhancer elements, bridging them to the pre-initiation complex at the promoter element. However, due to the complex's large size, structural flexibility, and intricate interactions, the mechanism by which Mediator recognises TFs remains poorly understood. To advance our understanding of TF-Mediator crosstalk, we investigated the interaction between TFs, the Mediator complex, and its kinase modules. Using Alphafold-based screening combined with *in vitro* pulldown assays, we identified potential TF-binding sites within the tail modules of Mediator complex. Additionally, we obtained near atomic resolution structures of Mediator, MKMCDK8 and MKMLCDK19 in their native states. Ongoing structural studies aim to elucidate how multiple TFs are recognised by Mediator, providing fundamental insights into how enhancer-bound TFs recruit CRs for transcriptional regulation.

Other Information:
Keywords: Mediator complex, Mediator kinase module, Pre-initiation complex, RNA Pol II, Protein-protein-interaction, Enhancer-promoter contact, Effector domain, DNA-binding domain, Transcription factor, Cryo-EM, Transcription regulation, Chromatin, Nucleosome

41

The Protein NanoHedra database (PNHdb): An innovative database for the design of novel symmetric protein architectures via deep learning methods

Laura Perez-Chirinos, Pablo San Segundo-Acosta, Roger Castells-Graells

Structural Biology Programme, Spanish National Cancer Research Centre (CNIO), Melchor Fernández Almagro 3, 28029 Madrid, Spain

Protein-based nanomaterials have emerged as powerful tools in the field of synthetic biology, spanning applications from nanotechnology to biomedical engineering. Protein nanohedra are three-dimensional geometric protein assemblies, i.e. tetrahedrons, octahedrons, icosahedrons. These nanohedra have potential applications in various fields, including their use as molecular containers for drug delivery or as imaging agents for diagnostics, among others. Computational methods have advanced significantly in recent years to design amino acid sequences that fold into desired protein structures, a process that recent innovations in artificial intelligence (AI) are making more feasible with time by enabling the rapid design of protein assemblies. However, the field lacks a centralized repository of natural and designed nanohedra to facilitate systematic analysis and novel design strategies for developing these self-assembling architectures. To address this gap, we are developing a comprehensive database that includes all available information of protein nanohedra, named the Protein NanoHedra database (PNHdb). This database compiles all available sequences of designed and natural protein nanohedra, providing detailed structural information, amino acid sequences, symmetry of the assemblies, biophysical properties, and design strategies. Furthermore, we are using the PNHdb to develop new deeplearning- based design tools by training neural networks to create novel nanohedra architectures. These novel designs will be used to encapsulate DNA and RNA molecules, offering the desired features to enhance cellular uptake, targeted delivery, and protection against enzymatic degradation, making these architectures ideal candidates for gene therapy, RNA vaccines, and programmable nanocarriers. Through a combination of data curation, deep-learning models, and new structures, the PNHdb will open new frontiers in synthetic biology, and biomedical engineering.

42

A filamentous scaffold for gene regulation

Tim Rasmussen[1,2], Jannik Küspert[3], Lars Schönemann[1], Dietmar Geiger[3], Bettina Böttcher[1,2]

1. University of Würzburg, Rudolf Virchow Centre, 97080 Würzburg, Germany
2. University of Würzburg, Biocentre, Chair of Biochemistry II, 97074 Würzburg, Germany
3. University of Würzburg, Julius-von-Sachs-Institute for Biosciences, Department of Molecular Plant Physiology and Biophysics, 97082 Würzburg, Germany

Proteins of the Drosophila behaviour/human splicing (DBHS) family are involved in many aspects of gene regulation and maintenance like transcription, splicing and DNA repair. The three known members of this family in humans, Non-POU domain-containing octamer-binding protein (NONO), splicing factor proline/glutamine rich (SFPQ), and paraspeckle protein component 1 (PSPC1), form homoand heterodimers to fulfil these functions by mediating contacts between RNA, DNA, and other protein factors. The dimers can further dynamically oligomerise through α-helical coiled-coils to larger aggregates, which is crucial for many functions of DBHS proteins. While the atomic structures of the dimers are established, the native arrangement in higher oligomers was unknown. Here we present the structure of a filamentous NONO/SFPQ heterooligomer from Cricetulus griseus resolved by cryo-EM. Globular heterodimer domains are alternating on both sides of a strand that is stabilized by an interdigitating network of coiled-coil interactions. Two of these strands assemble into a double strand with only few interactions between them. The globular domains of SFPQ face the counter strand and form a groove while those of NONO face outwards. The different environments of NONO and SFPQ in the filament provide the basis for a differential functionality.

Other Information:
bioRxiv: doi: https://doi.org/10.1101/2024.10.07.617013

43

Decoding Chikungunya Virus Membrane-Associated Replication

Rhian Jones[1], Vasiliya Kril[2], Michael Hons[3], Celine Amadori[2], Claire Zimberger[1], Laurine Couture[2], Yara Bouery[2], Julien Burlaud-Gaillard[4], Andrei Karpov[1], Denis Ptchelkine[1], Alexandra L. Thienel[5], Beate M. Kümmerer[5], Ambroise Desfosses[6], Philippe Roingeard[4], Laurent Meertens[2], Gabriel Bragagnolo[1], Rocío Arranz[7], Ali Amara[2] & **Juan Reguera[1]**

1- Aix-Marseille Université, CNRS, AFMB UMR 7257, ViMaC team ERL-INSERM U1324, Marseille, France
2- Université de Paris-Cité, Biology of Emerging Viruses Team, INSERM U944/ CNRS UMR 7212, Hôpital Saint Louis, Paris, France
3- EMBL, Grenoble, France
4- Université de Tours, INSERM U1259 MAVIVH et Plateforme IBiSA de Microscopie Electronique, Tours, France
5- Institute of Virology, Medical Faculty, University of Bonn, Germany
6- Institut de Biologie Structurale, Université Grenoble Alpes, CEA, CNRS, IBS, Grenoble
7- National Center of Biotechnology, CSIC, Madrid, Spain

Positive-sense single-stranded RNA viruses, such as coronaviruses, flaviviruses and alphaviruses, carry out transcription and replication inside virus-induced membranous organelles within host cells. The remodeling of the host-cell membranes for the formation of these organelles is coupled to the membrane association of viral replication complexes and RNA synthesis. These viral niches allow for the concentration of metabolites and proteins for the synthesis of viral RNA, which prevents the detection of this RNA by the cellular innate immune system. I will present the cryo-electron microscopy structures of non-structural proteins 1 and 3 (nsP1 and 3) of the alphavirus chikungunya virus, which are responsible for RNA capping and membrane binding of the viral replication machinery (nsP1) and the interaction with host factors and generation of cytoplasmic non-membranous organelles (nsP3). nsP1 structure shows the enzyme in its active form, assembled in a monotopic membrane-associated dodecameric ring. The structure shows how the complex formation couples the membrane binding, oligomerization and allosteric activation of the capping enzyme. The nsP3 structure shows tubular macro-assemblies present in both viral replication complexes and in honeycomb-shaped cytoplasmic granules which gather host and viral proteins and RNA. Our results provide high-resolution information about the membrane association of the replication machinery of positive-sense singlestranded RNA viruses, and the structural basis of the generation of membrane and non-membrane viral organelles which control the spatiotemporal organization of viral action for taking over the cell. Here we provide a new understanding of alphavirus infection opening up new avenues for the generation of antivirals.

44

Assessing AlphaFold3 Models of Transposition Complexes Through Cryo-EM

Irene Rizzuto[1,*], Mercedes Spínola-Amilibia[1,*] & Ernesto Arias-Palomo[1]

1. Centro de Investigaciones Biológicas Margarita Salas, CSIC, Madrid, Spain.
*. These authors contributed equally / ✉earias @ cib.csic.es

Transposition is a fundamental driver of genome evolution, enabling the movement of discrete DNA segments (transposons) within genomes. This process can modulate gene expression and contributes to the spread of antibiotic resistance and virulence factors. We recently determined the cryo-electron microscopy (cryo- EM) structures of the IS21 transposon, a widespread mobile genetic element that encodes IstA, a transposase, and IstB, a AAA+ ATPase essential for DNA transposition. The reconstructions of these factors in the pre- and posttransposition states revealed key insights into the architecture and conformational dynamics of the transpososome. In parallel, we evaluated one of the newest structure prediction engines, AlphaFold3 (AF3), to explore its capacity to model these intricate protein–DNA assemblies. Our findings show that while AF3 excels at predicting individual monomeric domains and select oligomeric arrangements, it struggles to capture complex assemblies, conformational changes, and higher-order interactions critical for transposon function. Overall, the comparison between the AF3 predictions and our experimental data highlight some of the strengths and limitations of current computational tools.

45

Structural-based engineering of virus-like particles formed by endogenous human Gag-like proteins

San Segundo-Acosta, P., López-Chirinos, L., Castells-Graells, R.

Structural Biology Programme, Spanish National Cancer Research Centre (CNIO), Madrid, Spain

A significant part of the human genome originates from selfish genetic elements that maximize their replication and spread. In mammals, the most common selfish elements are retrotransposons, which are classified into long-terminal-repeat (LTR) and non-long-terminal-repeat (non-LTR) retrotransposons. LTR retrotransposons include endogenous retroviruses (ERVs) and other families from the Ortervirales order of reverse-transcribing viruses, such as Metaviridae, Belpaoviridae, and Pseudoviridae. These elements encode gag and polymerase (pol) genes, both flanked by LTRs.

Gag genes, which encode structural proteins, have been domesticated for important host functions, and two dozen domesticated Gag-like proteins exist in humans . Several of these proteins have been shown to form virus-like particles (VLPs) that play crucial roles in cellular communication and neuronal plasticity.

However, although many other Gag-like proteins also preserve complete capsid domains, their potential VLP structure and function remains unclear.

In our group, we aim to use single-particle cryo-EM to obtain the structures of VLPs formed by several domesticated human gag-like proteins and to employ AIdriven protein design tools to engineer these assemblies for biomedical applications.

46

Cryo-EM uncovers a sequential mechanism for RNA polymerase I pausing and stalling at abasic DNA lesions

Alicia Santos-Aledo[1], Adrián Plaza-Pegueroles[1], Marta Sanz-Murillo[1], Federico M. Ruiz[1], Peini Hou[2], Jun Xu[2], David Gil-Cartón[3,4,5], Dong Wang[2], Carlos Fernández-Tornero[1]

1 - Centro de Investigaciones Biológicas Margarita Salas (CIB), CSIC, 28040 Madrid, Spain
2 - Skaggs School of Pharmacy and Pharmaceutical Sciences, UCSD, La Jolla, CA 92093, USA
3 - Basque Resource for Electron Microscopy, Leioa, Spain
4 - Instituto Biofisika (UPV/EHU, CSIC), University of the Basque Country, 48940, Leioa, Spain
5 - Ikerbasque, Basque Foundation for Science, 48009, Bilbao, Spain

Eukaryotic cells withstand over 50,000 DNA lesions per day, of which the most abundant are abasic sites, representing 20% of all lesions. Their timely detection is key to maintain genomic integrity and cell survival, but many of these mechanisms remain elusive. In scanning ribosomal DNA to produce the rRNA precursor, RNA polymerase I (Pol I) is particularly relevant in the detection of DNA lesions, as it is responsible for over half of the transcriptional activity in growing cells. We solved nine cryo-electron microscopy structures to study the sequential mechanism of yeast Pol I pausing and stalling at abasic sites, and supported those results with invitro transcriptional studies. Our results show that slow nucleotide addition opposite a templating abasic site is supported by base sandwiching between the RNA 3'-end and the Pol I bridge helix. Additionally, the encounter with the abasic lesion induces opening of the Pol I cleft: this flexibility supports A12-Ct access into the active center to stimulate RNA cleavage, as well as dissociation from DNA. We also report the atomic structure corresponding to nucleotide addition opposite the lesion, where an early translocation intermediate is induced. There, DNA bases in the hybrid tilt to form hydrogen bonds with the newly-added RNA base, in a manner different from previously-described RNA polymerase paused states. While in this state nucleotide addition is strongly disfavoured, intrinsic Pol I RNA cleavage activity acts as a failsafe mechanism to minimize lesion bypass. These results reveal mechanisms leading to prolonged Pol I stalling after nucleotide addition opposite Ap sites, which is distinct from arrest by bulky lesions and from Pol II blockage at abasic sites.

47

The Biochemistry and Structural Biology Unit at IEO, a platform for protein production and structural characterization

Giuseppe Ciossani[1], Silvia Monzani[1] and **Luigi Scietti[1]**

1. Biochemistry and Structural Biology Unit, Department of Experimental Oncology, IRCCS European Institute of Oncology (IEO), Via Adamello 16, 20139 Milan, Italy.

The Biochemistry and Structural Biology Unit (BSU) at the European Institute of Oncology (IEO – Milan, IT) offers a comprehensive range of services in protein biochemistry, biophysics, and structural biology. We work closely with researchers to facilitate the production and structural characterization of protein macromolecular complexes. The BSU employs optimized protocols and SLIC-based plasmid libraries for recombinant protein expression in various hosts, including E. coli, insect cells (Sf9 and High5), and suspension HEK293 cells. Together with multiple AKTA systems, this forms a robust platform for protein expression and purification. Our biophysical instruments encompasses SEC-SLS, mass-photometry, micro-ITC, BLITz, and FP plate readers. We have a mosquito nanoliter dispenser for crystallization screenings, all the necessary equipment for hit optimization, regular access to synchrotron facilities and computational resources for structure determination.
Additionally, we provide training and support in cryoEM, from sample preparation to data processing. We recently established a cryoEM lab, including a glow discharger a Vitrobot Mark IV and a BioComp gradient maker. We have regular access to 200 kV and 300 kV cryoEM for screening and data collection. For data processing, we operate a multi-GPU workstation that will be soon integrated with a multi-GPU high-performance cluster.

48

Biophysical Studies of LINE-1 ORF1p Protein and Its Binding Partners

Nerea Sorarrain de Pedro and Guillermo Abascal-Palacios

Instituto Biofisika (UPV/EHU-CSIC)

LINE-1 (L1) retrotransposons constitute approximately 17% of the human genome and play a crucial role in genome plasticity and evolution. The ORF1p protein, a key component of the L1 retrotransposition machinery, is an RNA-binding protein with nucleic acid chaperone activity, facilitating the formation of ribonucleoprotein particles (RNPs). L1 activity has been associated with diseases including cancer and age-related disorders, making the study of its regulatory mechanisms highly relevant. However, the molecular mechanisms underlying ORF1p interactions with nucleic acids and other cellular factors remain poorly understood.
Our study focuses on the interaction between ORF1p and TAR DNA-binding protein 43 (TDP-43), an RNA-binding protein implicated in neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). TDP-43 has been suggested to modulate L1 activity, linking retrotransposition to neurodegeneration. We purified full-length TDP-43, its truncation (1-266), mutants S48E and F147/149L (designed to increase its binding to ORF1p), as well as full-length ORF1p and its RRM and CTD domains. We are also studying ORF1p's nucleic acid binding to determine its mode of interaction.
To gain molecular-level insights into these interactions, we are using a multidisciplinary approach that includes NMR spectroscopy and cryo-electron microscopy (cryo-EM), which would allow us to map binding interfaces at high resolution and to clarify the mechanisms of ORF1p interactions and their regulation.
Our findings will contribute to understanding how ORF1p interacts with nucleic acids and cellular proteins, shedding light on the molecular mechanisms governing L1 retrotransposition and its potential regulation by RNA-binding proteins such as TDP-43. Given the involvement of both L1 and TDP-43 in human disease, unraveling their interplay may provide new insights into pathological mechanisms and potential therapeutic targets.

49 eSPAN Analysis of Cohesion Establishment during DNA Replication

Marta Velasco-Díez[1], Ivan Psakhye[2], Dolores Jurado-Santiago[1], Katsuhiko Shirahige[3], Dana Branzei[2] & Rodrigo Bermejo[1]

1. Margarita Salas Center for Biological Research (CIB-CSIC), Madrid, Spain
2. IFOM ETS, the AIRC Institute of Molecular Oncology, Milan, Italy
3. Institute for Quantitative Biosciences, The University of Tokyo, Tokyo, Japan

DNA replication is the process by which cells generate identical copies of their genome, after which, the two identical sister chromatids generated are held together by the cohesin complex. Proper establishment and maintenance of sister chromatid cohesion are critical for accurate chromosome segregation during mitosis.
Although cohesin can associate with chromatin throughout the cell cycle, cohesion is established during DNA replication. As replication forks progress, cohesin transitions from encircling a single DNA molecule (un-replicated DNA) to entrapping both sister chromatids.
Several replisome-associated proteins participate in cohesion establishment. They are thought to promote siter chromatid entrapment either through direct engagement of the cohesin ring or by facilitating transitions at newly synthesized DNA chains. Nonetheless, the molecular mechanisms mediated by which replisome-associated factors promote cohesion establishment remain poorly understood.
In this study, we aim at deepening our understanding on cohesion establishment during replication, by analyzing sister-chromatid interfacing with cohesin in strandresolved manner. We plan to use budding yeast eSPAN (enrichment and sequencing of protein-associated nascent DNA), a technique that allows strand-specific analysis of protein binding to newly synthesized DNA, to analyze cohesin dynamic association to nascent duplexes in contexts deficient in replisome-associated cohesion establishment factors and gain insight on their molecular functions in sister chromatid entrapment.
Our preliminary findings evidence cohesin enrichment at sites of active replication and an equal association of cohesin complexes to leading and lagging nascent duplexes, hence validating eSPAN as a useful tool to study strand-resolved cohesin dynamics upon interfacing with replication forks.

50 SRSF1 in Action: Mapping the Spatial Choreography of pre-mRNA Splicing

André Ventura-Gomes, Laura Grzegorzek, Célia Carvalho, Maria Carmo-Fonseca, Pedro Prudêncio

Gulbenkian Institute for Molecular Medicine, Av. Professor Egas Moniz, 1649-028 Lisbon, Portugal

Serine/arginine splicing factor 1 (SRSF1) is a key regulator of pre-mRNA splicing, modulating splicing decisions according to the context and location of its binding sites. By recognizing exonic splicing enhancers, SRSF1 promotes the recruitment of spliceosome components and facilitates exon inclusion. Despite extensive studies, the spatial dynamics of SRSF1 and its interactions within the nuclear environment remain incompletely understood. Here, we employed APEX2 proximity labeling coupled with mass spectrometry to investigate changes in the SRSF1 interactome in human cells following inhibition of transcription or pre-mRNA splicing. We used stable cell lines expressing APEX2-tagged SRSF1 and PML (PMID: 35182477). Cells were treated with DRB, a transcription inhibitor that prevents the formation of new elongation complexes, or with pladienolide B (PlaB), a splicing inhibitor that blocks catalytic spliceosome assembly. After APEX2 activation, we isolated the biotinylated protein fraction and performed label-free quantitative mass spectrometry analysis. In untreated cells, this approach revealed 169 proteins specifically enriched in the proximity of SRSF1. Upon DRB treatment, SRSF1 showed increased association with nuclear speckle proteins and a decreased proximity to hnRNP proteins. Given that hnRNPs predominantly bind intronic regions of pre-mRNAs, this reduction likely reflects the depletion of newly synthesized transcripts. The increased interaction with nuclear speckle proteins is consistent with the accumulation of SRSF1 in enlarged nuclear speckles. In PlaB treated cells, early spliceosome assembly factors remained associated with SRSF1, whereas components of the catalytic spliceosome were depleted, consistent with PlaB's mechanism of action. Overall, our findings highlight the dynamic remodeling of the SRSF1 interactome in response to transcriptional and splicing perturbations, revealing how SRSF1's activity influences its interaction landscape

51 Structural analysis reveals the dynamic and diversity of HYERs

Han-Zhou Zhu[1,2], Zi-Xian Liu[1,2], Shouyue Zhang[1], Zhi-Hang Chen[1], Yun Liu[1], Longqi Li[1], Yun Yang[1], Jun-Jie Gogo Liu[1*]

Beijing Frontier Research Center for Biological Structure, Beijing Advanced Innovation Center for Structural Biology, State Key Laboratory of Membrane Biology, Tsinghua-Peking Center for Life Sciences, School of Life Sciences, Tsinghua University, Beijing 100084, China.

The Hydrolytic Endonucleolytic Ribozyme (HYER) is a sequence-specific, hydrolysis-based DNA endonuclease. Our previous work identified 9 GII HYERs with varying levels of activity. But the relationship between activity and structure remains unknown. Here, we obtain the cryo-EM map of HYER3-9, while for HYER3,4,5,6,8 get high resolution around 3-4 Å. Structural analysis indicated that the overall structure of HYER with different activity is similar, and those with high activity is more stable. The HYER can be classified into three different classes α, β and γ-type, according to the different activity. Structural detail analysis indicated that DI serve as a stable scaffold. The open-distance of DI is larger in γ HYER, and the α, β HYER share stable DV-DI interaction. The defective of catalytic core of β HYER might lead to the lower activity compared to α HYER. The analysis on ribosomal tree revealed that the evolutionary trajectory of HYER might refined the structural stability and activity. To enhance engineering capability, the end-recruiter and SPLIT-HYER approach demonstrates improved binding affinity and cleavage specificity on the DNA substrate. These findings enhance our understanding of the potential structural evolution of HYERs, and the engineering of HYERs offers a more versatile platform for diverse applications.

52 Temperature impact on the real-time kinetics of the human mitochondrial DNA Polymerase (Polγ)

Lyra Zumeta[1], Ismael Plaza-G. A.[1], Francisco J. Cao[1,2], Grzegorz L Ciesielski[3], Borja Ibarra[1]

1. IMDEA Nanociencia, C/ Faraday 9, 28049 Madrid, Spain
2. Universidad Complutense de Madrid, Departamento de Estructura de la Materia, Física Térmica y Electrónica
3. University of North Florida, USA

PolG is necessary for the replication of mitochondrial DNA, a process independent from nuclear replication. Recent studies suggest that mitochondria can reach temperatures of up to 50ºC, but this is the topic of some controversy and is being conested. By using optical tweezers coupled to a temperature controller, we measured the temperature-dependent real-time kinetics of the polymerase. Our results show that increasing temperatures below 37-40 ºC promote the velocity and the number of nucleotides added by the enzyme, whereas temperatures above 41 C start to become inhibitory. These results argue against models supporting mtDNA replication at 50 ºC and give evidence to validate previous models regarding DNA-enzyme mechanics previously developed in our group.

cnio - CaixaResearch
FRONTIERS
MEETINGS

Madrid 28th – 30th May 2025

Machines acting on DNA and RNA, a molecular mechanistic perspective

Previous CNIO Frontiers Meetings and CNIO Cancer Conferences

cnio stop cancer

2024

FRONTIERS IN IMMUNOMODULATION AND CANCER THERAPY: 2ND EDITION
16/10/2024 – 18/10/2024
Organisers: Luis Álvarez-Vallina, María Casanova-Acebes, Nabil Djouder, David Sancho, Gaia Trincucci.

MOLECULAR CHAPERONES IN CANCER AND PROTEIN QUALITY CONTROL
10/06/2024 – 12/06/2024
Organisers: Gabriela Chiosis, Nabil Djouder, Judith Frydman, Óscar Llorca, Paul Workman

2023

METASTASIS
06/11/2023 – 08/11/2023
Organisers: Julio Aguirre-Ghiso, Caroline Dive, Eva González-Suarez, Héctor Peinado, Manuel Valiente

GENOME ORGANISATION AND STABILITY
22/05/2023 – 23/05/2023
Organisers: Felipe Cortés, Óscar Fernández-Capetillo, Ana Losada, Andre Nussenzweig

2022

DIET, NUTRITION AND CANCER CELL METABOLISM
24/10/2022 – 26/10/2022
Organisers: Nabil Djouder, Nikla Emambokus, M. Carmen Fernández-Agüera, Valter Longo, Marcos Malumbres

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

CNIO FRONTIERS MEETINGS and CNIO CANCER CONFERENCES

Machines acting on DNA and RNA, a molecular mechanistic perspective

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: Óscar 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
Organisers: Manuel Hidalgo, Alberto Bardelli, Lillian Siu, Josep Tabernero

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
Organisers: Francesco Gervasio, Ermanno Gherardi, Daniel Lietha, Giulio Superti-Furga

CNIO FRONTIERS MEETINGS and CNIO CANCER CONFERENCES

Machines acting on DNA and RNA, a molecular mechanistic perspective

2011

RECAPTURING PLURIPOTENCY:
LINKS BETWEEN CELLULAR REPROGRAMMING AND CANCER
07/11/2011 - 09/11/2011
Organisers: Maria A. Blasco, Konrad Hochedlinger, Manuel Serrano, Inder Verma

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
Organisers: Toren Finkel, David M. Sabatini, Manuel Serrano and David A. Sinclair

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
Organisers: Elaine Fuchs, Maria A. Blasco, Eduard Batlle and Mirna Pérez-Moreno

2008

SIGNALLING UPSTREAM OF mTOR
03/11/2008 - 05/11/2008
Organisers: Dario R. Alessi, Tomi P. Mäkelä and Montserrat Sánchez-Céspedes

STRUCTURE AND MECHANISMS OF ESSENTIAL COMPLEXES FOR CELL SURVIVAL
23/06/2008 - 25/06/2008
Organisers: Niko Grigorieff, Eva Nogales and Jose María Valpuesta

DEVELOPMENT AND CANCER
04/02/2008 - 06/02/2008
Organisers: Konrad Basler, Ginés Morata, Eduardo Moreno and Miguel Torres

CNIO FRONTIERS MEETINGS and CNIO CANCER CONFERENCES

Machines acting on DNA and RNA, a molecular mechanistic perspective

2007

LINKS BETWEEN CANCER, REPLICATION STRESS AND GENOMIC INTEGRITY
05/11/2007 - 07/11/2007
Organisers: Oskar Fernández-Capetillo, Jiri Lukas, Juan Méndez and André Nussenzweig

MYC AND THE TRANSCRIPTIONAL CONTROL OF PROLIFERATION AND ONCOGENESIS
11/06/2007 - 13/06/2007
Organisers: Robert N. Eisenman, Martin Eilers and Javier León

MOLECULAR MECHANISMS IN LYMPHOID NEOPLASM
19/02/2007 - 21/02/2007
Organisers: Elias Campo, Riccardo Dalla-Favera, Elaine S. Jaffe and Miguel Angel Piris

2006

TELOMERES AND TELOMERASE-CNIO / JOSEF STEINER CANCER CONFERENCE
13/11/2006 - 15/11/2006
Organisers: Maria A. Blasco and Jerry Shay

MEDICINAL CHEMISTRY IN ONCOLOGY
02/10/2006 - 04/10/2006
Organisers: Fernando Albericio, James R. Bischoff, Carlos García-Echeverria and Andrew Mortlock

INFLAMMATION AND CANCER
22/05/2006 - 24/05/2006
Organisers: Curtis Harris, Raymand Dubois, Jorge Moscat and Manuel Serrano

PTEN AND THE AKT ROUTE
08/05/2006 - 10/05/2006
Organisers: Ana Carrera, Pier Paolo Pandolfi and Peter Vogt

2005

CANCER AND AGING
07/11/2005 - 09/11/2005
Organisers: Maria A. Blasco, Kathy Collins, Jan Hoeijmakers and Manuel Serrano

MAP KINASES AND CANCER
30/05/2005 - 01/06/2005
Organisers: Philip Cohen, Roger Davis, Worcester, Chris Marshall and Ángel Nebreda

ANIMAL TUMOUR MODELS AND FUNCTIONAL GENOMICS
07/03/2005 - 09/03/2005
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

CNIO FRONTIERS MEETINGS and CNIO CANCER CONFERENCES

Machines acting on DNA and RNA, a molecular mechanistic perspective

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

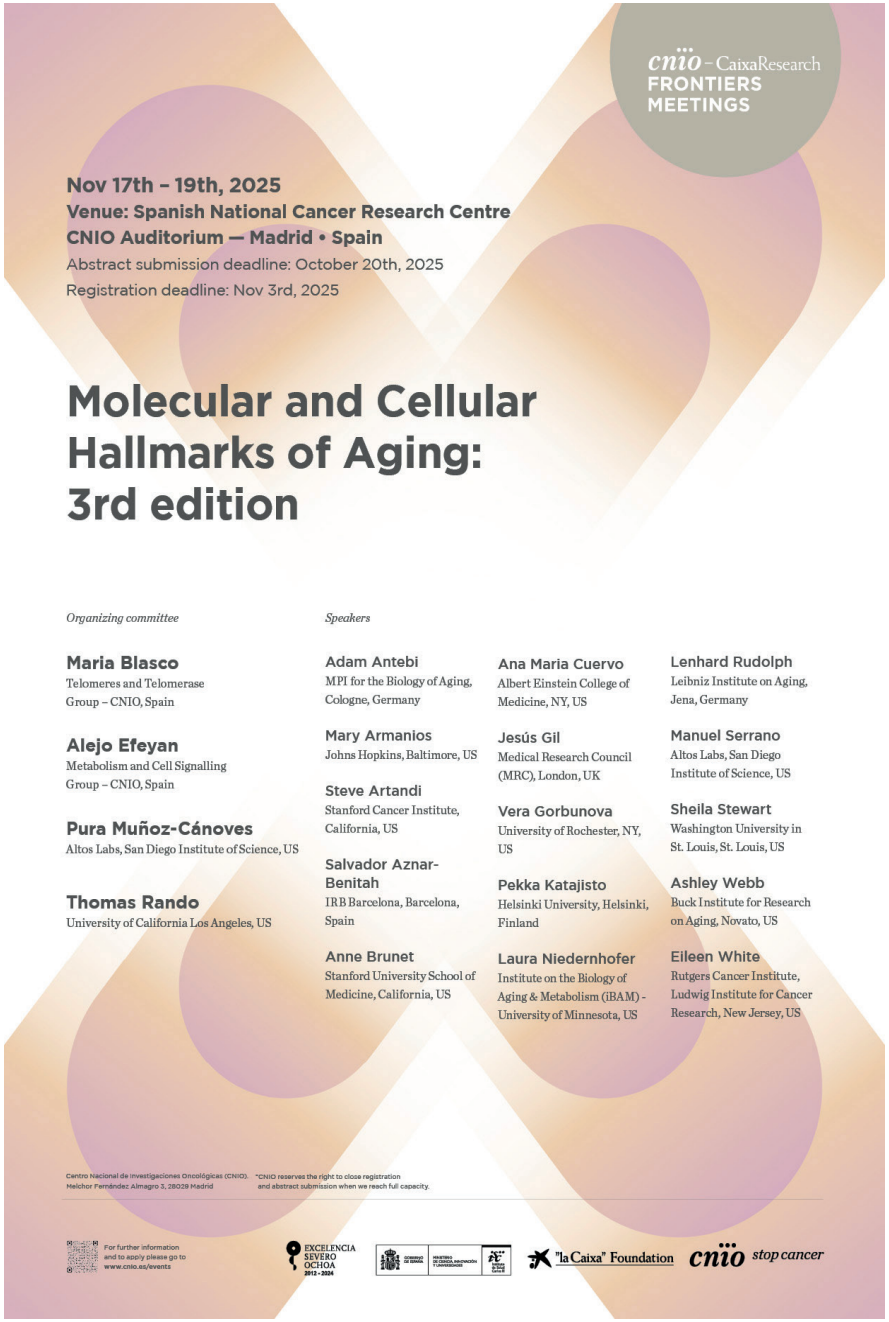
TARGETED SEARCH FOR ANTICANCER DRUGS
17/03/2003 - 19/03/2003
Organisers: Amancio Carnero and David H. Beach

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



2026

Metabolism and cancer: insights from obesity and beyond
MAY 20th - 22th, 2026

Venue:
Spanish National Cancer Research Centre – CNIO Auditorium, Madrid, Spain

Organizing committee:
Roger Davis, Umass Chan Medical School, US
Nabil Djouder, Spanish National Cancer Research Centre-CNIO, Spain
Alejo Efeyan, Spanish National Cancer Research Centre-CNIO, Spain
Miguel A. Quintela, Spanish National Cancer Research Centre-CNIO, Spain
Guadalupe Sabio, Spanish National Cancer Research Centre-CNIO, Spain

cnio - CaixaResearch
FRONTIERS
MEETINGS

Madrid 28th – 30th May 2025

Machines acting on DNA and RNA, a molecular mechanistic perspective

Notes

Machines acting on DNA and RNA, a molecular mechanistic perspective

cnio stop cancer

Notes

Machines acting on DNA and RNA, a molecular mechanistic perspective

Notes

Notes

Machines acting on DNA and RNA, a molecular mechanistic perspective

Notes

Notes

Machines acting on DNA and RNA, a molecular mechanistic perspective

Notes

Notes

Machines acting on DNA and RNA, a molecular mechanistic perspective

Notes

Notes

Machines acting on DNA and RNA, a molecular mechanistic perspective

Notes

Notes

Machines acting on DNA and RNA, a molecular mechanistic perspective

Notes

cnio - CaixaResearch
FRONTIERS
MEETINGS

Madrid 28th – 30th May 2025

Machines acting on DNA and RNA, a molecular mechanistic perspective

Centro Nacional de Investigaciones Oncológicas (CNIO)
Spanish National Cancer Research Centre
Melchor Fernández Almagro, 3
28029 Madrid, Spain
www.cnio.es

Coordination and edition:
Mercedes Moro, CNIO, Madrid, Spain
Production of art and design by Gedosol, S.L.
Photographic archive CNIO

Printed in Spain

#CFM_MolMech
@CNIOStopCancer
@CaixaResearch

cnio stop cancer

cnio stop cancer

For more information about
our events
www.cnio.es/en/eventos

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.

#CFM_MolMech

@CNIOStopCancer **@CaixaResearch**

EXCELENCIA SEVERO OCHOA 2012 - 2024

GOBIERNO DE ESPAÑA
MINISTERIO DE CIENCIA, INNOVACIÓN Y UNIVERSIDADES
Instituto de Salud Carlos III

cnio stop cancer

MCE MedChemExpress

"la Caixa" Foundation