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Écologie et Environnement
CONFÉRENCES
JACQUES-MONOD

Instituts thématiques

Inserm
Institut national
de la santé et de la recherche médicale

Roscoff (France), 9-13 September 2024

TRANSCRIPTION AND GENOME MAINTENANCE

Transcription et Maintenance du génome

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Rapport sur la Conférence
Conference Report

French summary/Résumé en français

La conférence Jacques Monod "**Transcription et Maintenance du genome**", s'est tenue à Roscoff du 9 au 13 septembre 2024. Cette conférence organisée Gaëlle Legube (Présidente) et Hourra Merrick a réuni un total de 90 participants du monde entier (France, Europe, USA, Israel, …), dont les orateurs invités et 66 participants (étudiants en thèse, post-doctorants et chefs d'équipes).
Au cours de cette conférence les participants ont discuté des avancées les plus récentes concernant la fonction des ARN dans l'instabilité du génome, et en particulier 1) du rôle des hybrides ARN :ADN, 2) des conflits entre les machineries de réplication-transcription comme source d'instabilité du génome, 3) du rôle de l'instabilité du génome générée par la transcription dans le cancer et les maladies, 4) du rôle des ARNs et de la transcription dans la réponse aux dommages à l'ADN, 5) du rôle des ARNs et de la transcription dans la dynamique et l'organisation des chromosomes et 6) de leur rôle dans la maintenance des télomères.

Grâce à l'implication des participants, des orateurs invités (24 présentations) et des orateurs sélectionnés suite à leur candidature (17 présentations), la conférence a été un véritable succès et une occasion unique d'échanger sur les concepts qui sous-tendent l'instabilité du génome générée par l'activité transcriptionnelle, sur les technologies utilisées et leurs limites, et sur le rôle de cette instabilité dans les maladies humaines.

The Jacques Monod Conference **"Transcription and Genome maintenance"** was held in Roscoff, France on September 9-13, 2024. This conference, organized by Gaelle Legube (Chair) and Houra Merrikh (Vice-chair) brought 90 participants, including the invited speakers and 66 applicants (PhD students, postdocs and a high number of PIs) from all over the world (France, rest of Europe, USA, Israel). The opening lecture was supported by EMBO, and delivered by Andres Aguilera, an EMBO member. The ARC foundation also supported the meeting and especially the session entitled "Transcription-induced genetic instability and RNA in cancer and diseases".

Aim of the Conference. The conference followed the one that took place in September 2019 in Roscoff on "Genome instability: when RNA meets chromatin" organized by Andres Aguilera (Chair) and Gaelle Legube (Vice-chair). The aim was to provide an update on this rapidly expanding field, on the technologies that are used to study RNA:DNA hybrids, and genome instability at the genomic scale, and to discuss the role of transcription-induced genome instability in disease, a focus that was not present at the previous edition of the meeting.
Of importance, this topic, centered on the role of transcription in inducing the DNA damage response and mutation/rearrangement on the genome, is usually hardly covered in DNA damage response and repair meetings.
The final aim of this Jacques Monod Conference was to bring together scientists studying the DNA damage response/genome stability and RNA biology to discuss these different topics to decipher the interconnections between the genome integrity, RNA metabolism and chromatin to help establish a continuity of this meeting in Europe and America, in such an important field of research. The conference came at the right time, as research in the last two decades placed replication and the DDR at the center of a complex interplay between DNA synthesis, transcription, RNA processing and epigenetics, with major consequences for genomic instability and diseases onset.

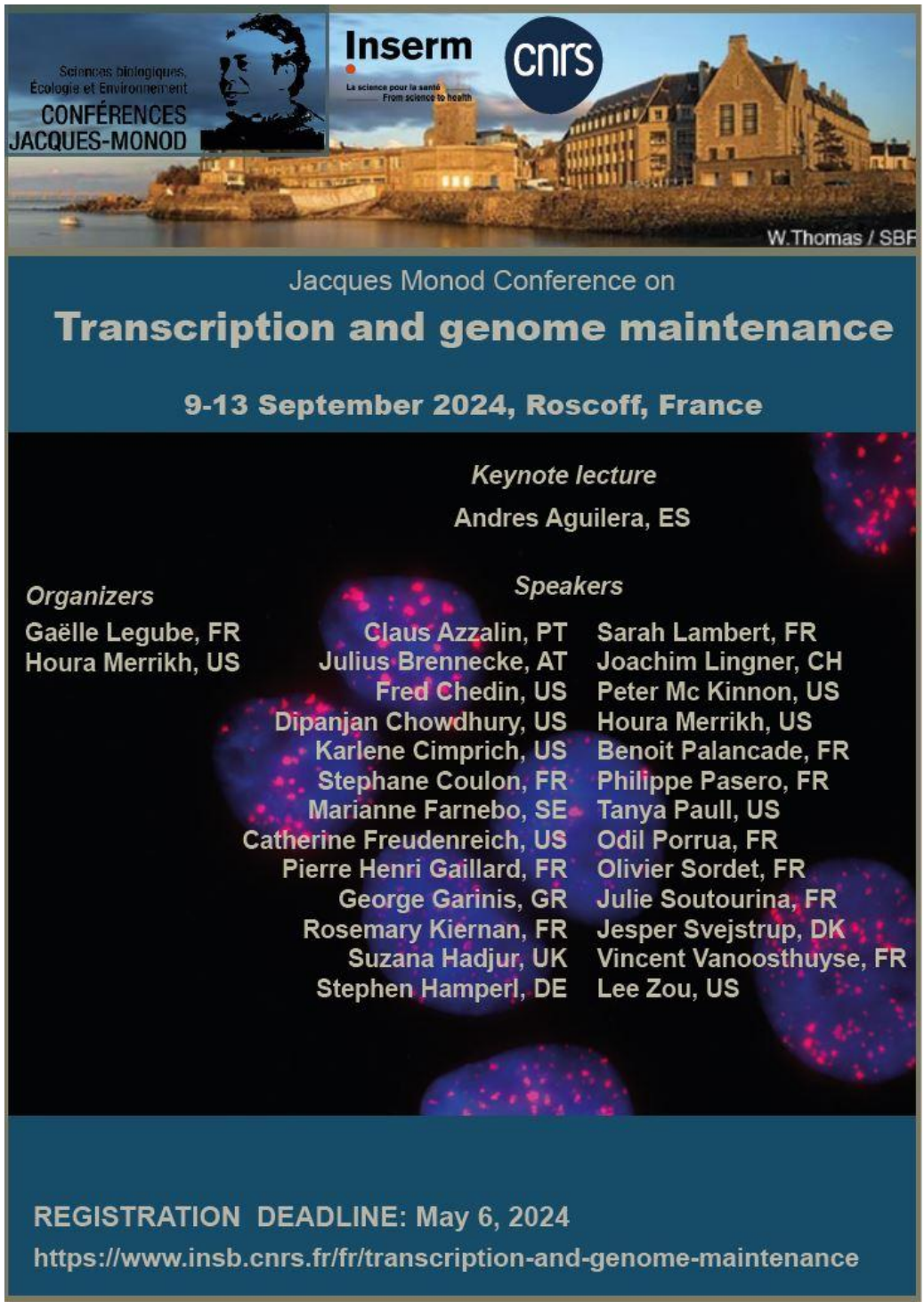
Conference overview. The conference started with the plenary lecture on Monday September 9th, given by Andres Aguilera, after welcome drinks and a dinner at the Gulf Stream Hotel. Lectures were given in the auditorium of the Station Biologique CNRS. The meeting entailed two poster sessions of 2h each, on Tuesday and Thursday afternoon. Each poster presenter had the occasion to present their poster during the "flash talk session" at the end of the morning session preceeding their poster session. Part of the Wednesday afternoon was devoted to an excursion to the "Ile de Batz" or free time. The conference ended on September 12th afternoon after a 1h discussion about the main highlights and potential next editions followed by a banquet at the Gulf stream. People departed early in the morning of September 13th

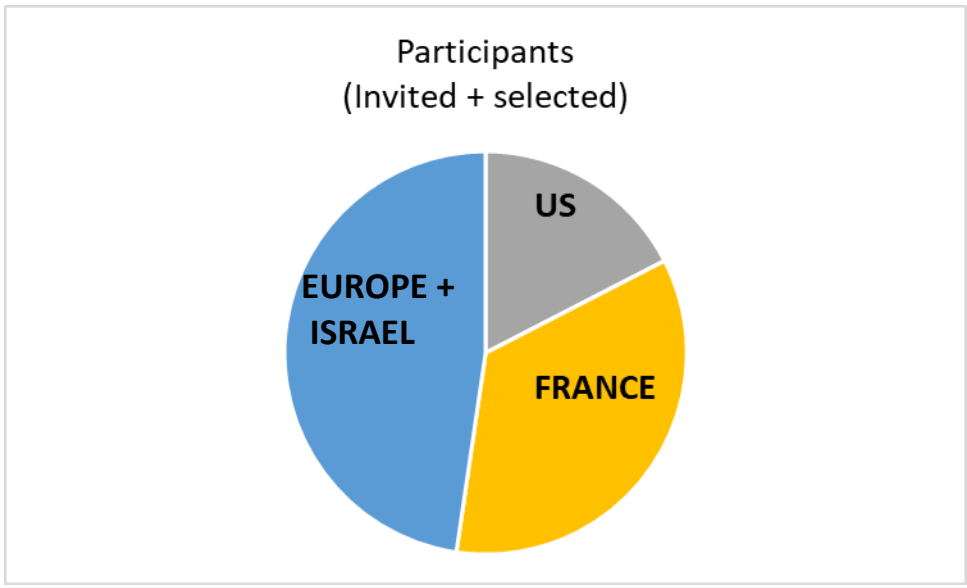
- 1 Plenary lecture (60 min)
- 6 sessions with a dedicated chair chosen among the invited speakers:
  - Session 1 – RNA:DNA hybrids: the good and the bad
  - Session 2 – Transcription-Replication conflicts as a source of genomic instability
  - Session 3 – Transcription-induced genetic instability and RNA in cancer and diseases
  - Session 4 – RNAs and transcription in the DNA damage response
  - Session 5 - RNAs and transcription in nuclear compartmentalization and chromosome dynamics
  - Session 6 – RNAs and transcription in telomere maintenance
- 24 presentations by invited speakers in sessions (20 min + 5 min for questions)
- 17 short presentations selected from abstracts (15 min + 5 min for questions)

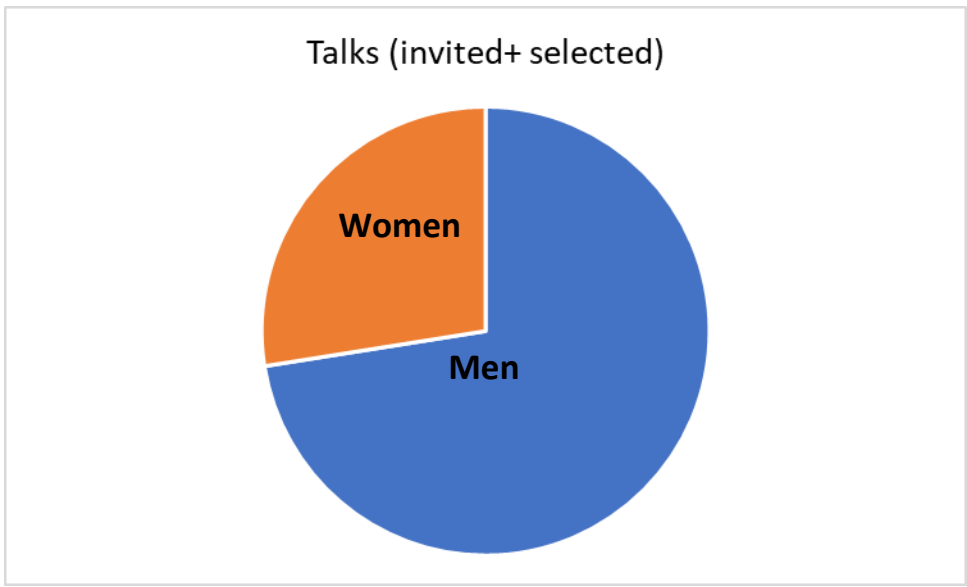
- Two poster sessions (2 hours each)
- Two Flash talk sessions (30min each)

All aspects of management of the conference and the food were more than satisfactory, thanks to the expert care of Nathalie Babic and her colleagues. Overall, the conference was a big success, with highly positive feedback of participants at both scientific and organization levels.

Conference statistics




Participants
(Invited + selected)
US
EUROPE +
ISRAEL
FRANCE


Talks (invited+ selected)
Women
Men

Scientific program

Scientific context

Chromosomes are incredibly long molecules that must be faithfully replicated and segregated at each cell cycle to provide genetic information to daughter cells. DNA replication and DNA repair need to be tightly linked to cell cycle progression, to maintain the integrity of our genome during cell divisions. To maintain genome integrity, cells have developed a complex signaling network that includes DNA repair and DNA damage tolerance pathways and cell cycle checkpoints collectively refereed as the DNA damage response (DDR). Failure to properly respond to DNA damage leads to genomic instability which is an underlining cause of various human genetics syndromes and associated with many age-related diseases, such as cancer or neurodegeneration.

Significant efforts over the past twenty years have focused on understanding how genome instability can arise. Among the mechanisms that generate instability, transcription emerged as a main source of DNA damage. The revolution of high throughput sequencing combined with advanced methodologies to map transcription, replication and DNA damage indeed revealed that genomic loci that display fragility are mainly those displaying transcriptional activity.

Moreover, although long overlooked, it is now well established that DNA does not systematically assemble into a canonical double helix (B-DNA), throughout the entire genome but can also accommodate other structures. Among these structures, RNA:DNA hybrids that form when the nascent RNA anneals back to the template DNA strand, also commonly referred as “R-loops”, have recently received a particular attention. R-loops are usually considered as toxic by-products of ongoing transcription generating structural obstacles that shall be removed to allow the processivity of RNA and DNA polymerases. Consequently, they have been shown to be a main source of DNA damage, especially DNA Double Strand Breaks, that when unrepaired or mis-repaired, are a real threat to genomic integrity. Accordingly, impaired R-loops removal pathways are emerging as a cause of multiples human diseases including cancers and neurodegenerative diseases.

However, counterintuitively, these RNA:DNA hybrids are also now recognized as displaying beneficial roles through their ability to regulate various processes. Among those beneficial roles, R-loops are key players in the regulation of transcription, chromatin structure, the maintenance of telomeres and the repair of DNA damage. Hence, rather than being simple detrimental roadblocks that must be eliminated, R-loops are also now considered as *bona fide* players of the genome activity in general and in particular of the mechanisms that ensure chromosome integrity.

The program of the meeting was designed to cover these topics, from the mechanisms that trigger instability on transcribing genomic loci including through the formation of R-loops, to the direct role of RNAs and transcription in the maintenance of the genome and in disease onset.

Plenary lecture (Chair: Gaelle Legube)

The keynote lecture was sponsored by EMBO and delivered by **Andres Aguilera**, who has been a pioneer in R-loop biology, discovering the function of several chromatin-bound complexes in R-loop regulation. He thus provided an excellent introduction to the meeting, detailing his major past and more recent discoveries on R-loop regulation and functions. Andres also discussed his lab's latest data on the function of Alyref in the regulation of RNA:DNA hybrids at DNA Double strand breaks.

Session 1 – RNA:DNA hybrids: the good and the bad (Session Chairs: Stephan Hamperl and Yea Lih Lin)

The first session was dedicated to RNA:DNA hybrids and R-loop, and more specifically how they can generate DNA damage and genome instability. This session also allowed to cover the technologies to detect them and to modulate their level in vivo to investigate their function, which is a major challenge in the field. **Fred Chedin** discussed the origin of DSB following R-loop accumulation. He presented his latest research, using high resolution mapping, showing that while both R-loop and DNA breaks appear in condition when intron are retained, yet DNA damage is not rescued by R-loop removal following RNAseH1 overexpression. This uncouples break formation from R-loop accumulation, challenging the common view on R-loop induced DSBs. **Vincent Vanoosthuyse** then presented a novel methodology to deliver controlled amount of RNAseH1 to living cells, using viral particles, called iCoRD, which behave as R-loop live sensors. Surprisingly, RNAseH1 delivery did not affected transcription and DRIP-seq and S9.6 Cut&Tag did not show any changes in R-loop levels, which questioned the commonly used RNAseH1 overexpression as a tool to modulate R-loop levels *in vivo*. **Domenico Libri** further presented a novel method for R-loop detection, called H-CRAC, relying on UV-mediated crosslink of RNAseH1 to its target loci, followed by purification and NGS sequencing. In yeast, H-CRAC signal was detected in transcribed genes, but not necessarily parallels the RNAPII signal on genes, indicating that pre-mRNA may be able to reanneal transiently, before being processed, while RNAPII is evicted. **Houra Merrick**, co-chair of the meeting was supposed to talk during this session but due to personal reason could not attend the meeting. She was replaced by one of her students attending the meeting, who presented that in contrast to *in vitro* experiment, where R-loop can act as replication origin, R-loops *in vivo* counteract origin firing. They identified AsnRS (asparagine tRNA ligase) as involved in preventing replication restart from R-loops. **Karlene Cimprich** discussed about the nature of the mysterious cytoplasmic DNA:RNA hybrids discovered by her team few years ago. She presented her latest unpublished work, using NGS technologies, showing that they arise from genes, but also enhancer and intergenic loci. She showed that upon SETX, BRCA1 and BRCA2 depletion, these cytoplasmic RNA:DNA hybrids accumulate but while SETX-sensitive hybrids accumulate from sites of convergent transcription, this is not the case for BRCA1/2 sensitive hybrids, suggesting multiples mechanisms for cytoplasmic hybrids accumulation. She described a mechanism allowing cytoplasmic formation, via the ORF1/2 retro-trancriptase activity. **Yvan Matic** discussed the consequences of increasing or decreasing the number of rDNA operons in *E.Coli*. Of interest he found that lower copy number, triggers slow growth and extended log phase, and massive DNA damage, due to the hyper-transcription of the remaining copies. **Philippe Pasero** discussed the role of DNA topology in R-loop and transcription replication conflicts, He discussed how TOP1 depletion trigger replication stress and the impact of RNAseH1 and H2 on the resection of arrested replication forks. Finally, **Alex Bishop** discussed how different R-loop mapping methods gives different results, and further focused on the R-loop arising in Ewing sarcoma, which display hyper transcription due to the EWS1-FLI1 fusion.

Session 2 – Transcription-replication conflict as a source of genome instability

Chairs: Olivier Sordet and Benoit Palancade

In this second session, we discussed how the collision between the transcription and the replication machineries can generate genomic instability. **Stephan Hamperl** describes a novel reporter assay in cells to monitor Transcription-replication conflicts (TRC). He showed that TRC are accompanied by nucleosome depletion and R-loop accumulation. He further showed a function for DOTL1 in di and tri-methylating H3K79 for transcription restart after such conflict. **Satish Thiyagarajan** showed a novel methodology, based on oxford nanopore sequencing to measure replication speed and site of pausing, and discussed how replication pauses at RFB present on rDNA in yeast. **Jessica Marinello** presented her work on how TOP1 poisons cause R-loops and replication-dependent DNA damage downstream on highly transcribed genes**. Yann Frey** presented how ZC3H4, a transcriptional regulator, regulates non coding RNA production thus preventing R-loops accumulation and DNA damage in human cells. **Jesper Svejstrup** briefly described their recent work on STK19 as a novel TC-NER factor. He then presented his work on the characterization of the interactome of modified RNAPII, using modified CTD peptides, and further focused on the function of S7 phosphorylation. **Marek Sebesta** discussed the mechanisms of transcription attenuation and condensation of RNA polymerase II by RECQ5.

Session 3 – Transcription-induced genetic instability and RNA in cancer and diseases (ARC labelled session) Chairs: Domenico Libri and Catherine Freudenreich

The third session focused on how diseases can arise from transcription induced genomic instability.

Lee Zou discussed his recent work indicating that the transcriptional program activated during Epithelial Mesenchymal transition (EMT) is associated with DNA damage due to R loop accumulation, especially when ATR is inhibited. ATR inhibition triggers not only increased R-loop and DNA damage but also reduces EMT efficiency, via an ATM dependent, damage induced, transcriptional repression of Snail1, an EMT gene. ATR is thus not only a sensor of transcriptional induced DNA damage, but also a protector of EMT genes. **Patrick Sung** showed that BRCA1/BARD1 complex strongly interacts with SETX, through the BRCT domain of BRCA1, and promotes R-loop resolution. **George Yacoub** discussed the role of ELOF1 and UVSSA in TC-NER. **George Garinis** presented exciting data showing a transfer of dsDNA containing extracellular vesicles from microglial cells to neurons in ERCC1 KO mouse cortex, spinal cord and cerebellar tissues. His data also suggest that R-loop are transferred and activate the proinflammatory response. **Hannes Lans** found in *C.elegans*, that UV sensitivity induced by the TC-NER mutations (such as UVSSA) is actually due to the lack of RNAPII clearance. TC-NER mutant that are not deficient in clearance are actually not sensitive to UV. He shows that the reason for UV resistance is that GG-NER can actually repair and compensate for TC-NER deficiency if it can access the breaks. **Odile Porrua** discussed the function of R-loop metabolism in the motor neurons of ALS4 patient. **Haziz El Hage** presented his work performed in the lab of David Tollervey, showing that RNAseH/nuclear exosome double mutant in budding yeast display increased R-loop at CUT (cryptic unstable transcripts) and SUT (stable unnonotated transcript) which further triggers genomic instability. **Olivier Sordet** presented his work on the Scan1 disease. They developed a SCAN1 model in U2OS and showed that endogenous transcriptionally induced DSB accumulate in G1 cells and not in S-phase. Finally, **Benoit Palancade** closed this session by discussing their screen for R-loop induced hyperrecombination. Their data suggest that in contrast to the common model,

most of the R-loop actually arise due to a DNA lesion, and do not contribute themselves to genome instability.

Session 4: RNAs and transcription in the DNA Damage Response-I&II. Chairs: Julie Soutourina, Patrick Sung, Odil Porrua

This session weas dedicated to the role of RNA and transcription in DNA damage repair.
The session started by a talk from **Dipanjan Chowdhury** who discussed an exciting work identifying cis regulatory elements on the 3'end of the MRE11 gene. He showed evidence that this element promotes DNA looping coordinating the transcription cycle in response to DNA damage. **Pierre Henri Gaillard** discussed his ongoing work on the function of Slx4, mysterious DNA helicase that can binds G4, D-loops, R-loops, recombination intermediates. **Alessandra Brambati**, coming from the lab of Agnel Sfeir, presented a new DNA repair pathway involving Pol-zeta dependent transcription form transcript RNA. **Marianne Farnebo** presented her work on RNAs in repair, and more specifically their recent observations that ASO (antisense oligo nucleotides) accumulate in nuclear condensates, which also accumulate and hyperactivate DNA damage response kinases, calling for caution when using this type of tool, both for basic research than therapeutic purposes. Then **Aline Marnef**, presented her data obtained in the lab of Gaelle Legube, on the biogenesis of RNA:DNA hybrids at DSBs. She showed that RNA polymerases are not recruited *de novo* at sites of damage, and RNA:DNA hybrids do not arise from de novo transcription, but from the hybridization of pre-existing RNA.
The follow-up of this session on Thursday morning started with a talk from **Kyle Miller** who presented exciting unpublished data on the fact that Cas9-induced breaks can in some instance activate transcription. Then **Julie Soutourina** presented their work on the function of the mediator complex during TC-NER. **Neus Visa** discussed the function of the exosome and in particular of DIS3 in TC-NER and transcription recovery after UV irradiation while **Jurgen Marteijn** describe their recent discovery of STK19 as a new member of the TC-NER pathway (also mentioned by Jesper Svetrup in his talk: this new member has been co-identified by four different lab this year). **Dong Wang** further provided structural insights on STK19 and UVSSA.

Session 5: RNAs and transcription in nuclear compartmentalization and chromosome dynamics. Chairs PH Gaillard and George Garinis

Julius Brennecke presented the piRNA pathway. He showed that Rhino (H3K9me3 binder) accumulate at the piRNA cluster and recruits RNAP2 to force transcription of the locus. He further presented their work on the pathway allowing to export these piRNA in the cytoplasm. **Catherine Freudenreich** presented new data on the function of APOBEC3A, which trigger damage on CAG repeat, especially in R-loop processing deficient conditions. **Gaelle Legube** presented that following DSB, the chromosome architecture is reorganized in the nucleus, leading to the formation of a novel chromatin compartment, the D-compartment, which is required to optimally activate the DNA damage response. The formation of the D-compartment depends on R-loops.
Christophe Chapard presented exciting data showing that transcription can actually also organize the yeast 3D genome, and can serve of "moving" loop extrusion barriers.

Session 6: RNAs and transcription in telomere maintenance. Chair Vincent Vanoosthuyse

Finally, the last session of the meeting was dedicated to the particular case of the contribution of RNAs in the integrity of telomeres, with two speaker talks (Claus Azzalin had to cancel his venue due to medical reasons).
Stephane Coulon presented his Pu-seq data allowing to determine by which polymerase telomeres are extended following sen1 depletion (R-loop accumulation) in yeast, while **Joachim Lingner** discussed the relationship between TERRA (a non-coding RNA associated to telomeres) and R-loop in ALT cancer cells.

Altogether, despite the last minute cancelation of some of the speakers (including the co-chair for personal family reason), the conference was a scientific success attending all comments and feedbacks of participants during and after the meeting. The participants also appreciated a lot the two flash talk sessions which gave them the opportunity to present very briefly their poster. The poster sessions were extremely well attended and of high quality.

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