Speakers' abstracts

(in order of appearance in the program)

Maria Carmo-Fonseca

Instituto de Medicina Molecular João Lobo Antunes, University of Lisbon, Portugal

From co-transcriptional splicing to genome instability

Since it became clear that introns are spliced out from precursor pre-mRNA molecules in the nucleus before mature mRNAs are exported to the cytoplasm, questions were raised about the timing of splicing. Does splicing start while RNA polymerase II is still transcribing? Is splicing a slow or a fast process? Is timing important to control the splicing reaction?

To visualize pre-mRNA splicing in living cells, we genetically inserted the binding sites for the MS2 bacteriophage coat protein in the intron of a reporter gene that was integrated in the genome of cells expressing the MS2 coat protein fused to GFP. We found that transcription and excision of short introns (1.3–1.4 kb) occurs in 20–30 s, which implies a splicing rate within a few seconds (Martin et al. 2013).

To further investigate the timing of pre-mRNA splicing relative to the position of RNA polymerase II (Pol II) at genome-wide level, we initiated a collaboration with Nick Proudfoot at the University of Oxford, who had developed a novel strategy for Native Elongation Transcript sequencing (NET-seq). We found an accumulation of transcripts mapping precisely to the 3' end of exons, as expected for intermediates formed after the first transesterification splicing reaction, indicating that splicing must occur within a stable complex formed between the spliceosome and Pol II (Nojima et al 2015, 2018). However, in mammalian cells we did not detect spliced products associated with polymerases transcribing the exon downstream of a 3' splice site. This may be because mammalian introns are long and rely on an exon definition splicing mechanism. To study cells from an organism with a distinct exon-intron architecture, we optimized the NET-seq approach to analyze nascent transcripts in the developing Drosophila embryo (Prudencio et al. 2020). We found widespread evidence for recursive splicing taking place shortly after Pol II transcribes past a recursive splice site within long introns. We also detected spliced products associated with polymerases transcribing the first 100 nucleotides of the downstream exon. Thus, our observations indicate that at least a subset of Drosophila introns are excised immediately after transcription of the 3' splice site. More recently, we analyzed long stretches of nascent RNA associated with Pol II by direct single-molecule sequencing and we found that splicing in human cells can also be completed right after the 3’ splice site is transcribed (Sousa-Luis et al. 2021).

Does it matter whether splicing is fast or slow? We still have no answer to this question. However, mutations in several splicing factors enhance the formation of R-loop structures whereby the nascent RNA invades the DNA duplex behind elongating Pol II. R-loops may interfere with DNA replication, repair, and transcription, thus compromising genome integrity and function. We therefore propose that fast splicing may act as a safeguard against R-loop mediated genomic instability.

References:

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Hervé Le Hir

Institut de Biologie de l'École Normale Supérieure, Paris, France

The multiple facets of the Exon Junction Complex

Hervé Le Hir is group leader at IBENS (Institut de Biologie de l'École Normale Supérieure, UMR8197). His team studies the life of mRNPs and particularly the role of EJCs (Exon Junction Complex). We notably explore the molecular mechanisms at play in the process of NMD (Nonsense-mediated mRNA decay), an mRNA quality control pathway. We combine various approaches including molecular biology, biochemistry, structural biology, transcriptomics, cellular biology and biophysics to isolate and dissect the action mode of RNA-protein complexes. We reconstituted and characterized several EJC and NMD RNA-protein complexes, we established EJC map by CLIP-seq and we imaged EJC proteins to study its role in mRNA localization. We also have a particular interest in the biophysical properties of RNA helicases that are small molecular motors essential for RNP dynamics.

Martin Dutertre

Institut Curie, Orsay, France

Regulation of intronic polyadenylation isoforms by genotoxic anticancer agents

Intronic polyadenylation (IPA), which generates short mRNA isoforms, is enriched in genes involved in the DNA-damage response (DDR), and IPA isoforms are widely regulated by genotoxic agents. However, little is known about the fate, translation and function of IPA transcripts. Here, we characterize IPA isoform regulation by two genotoxic anticancer agents, doxorubicin and cisplatin, by using several genome-wide approaches, and by analyzing several cell compartments and polysome fractions. First, by 3'-seq (RNA-seq focused on the 3'-end of polyA+ RNA) on whole cells, we show that doxorubicin mainly down-regulates, while cisplatin mainly up-regulates IPA isoforms relative to full-length mRNA. This effect of cisplatin is enriched in long genes, and correlates with a decrease in RNA polymerase II processivity (as shown by analysis of intronic reads in total-RNA-seq) and mRNA length (as shown by long-read RNA-seq). For both drugs, IPA regulation events are enriched in DDR and cell cycle-related genes. Second, 3'-seq analyses on several cell compartments reveal that doxorubicin mainly decreases IPA isoforms in the nucleus, but often increases IPA isoforms in the cytosol. Condition- and compartment-specific CLIP-seq (and 3'-seq) analyses indicate that the RNA-binding protein, HuR coordinates these nuclear and cytosolic levels of regulation. Third, 3'-seq analyses on polysomal fractions identify sets of IPA isoforms, that are either efficiently or inefficiently translated. They also reveal a set of 5'UTR-IPA isoforms, that terminate in the annotated 5' untranslated region of genes, but are associated with light polysomes and potentially encode micropeptides. Finally, using isoform-specific RNAi, we identify translated IPA (including 5'UTR-IPA) isoforms, that modulate cancer cell sensitivity to genotoxic drugs. These findings reveal diverse fates and translational outcomes of IPA isoforms regulated by genotoxic agents.

Jesper Q. Svejstrup

The Francis Crick Institute, London, United Kingdom

Institute of Cellular and Molecular Medicine, University of Copenhagen, Denmark

The transcription response to stress and DNA damage

The efficient production and correct processing of nascent RNA polymerase II transcripts is essential for life. Factors that affect transcription and mRNA splicing, including DNA damaging agents, can thus have a dramatic effect on gene expression and cell viability. Indeed, upon UV-irradiation, a slowdown of transcript elongation and restriction of gene activity to the promoter-proximal ~25 kilobases is observed. This is associated with a change in poly-adenylation site (PAS) selection, altering transcriptional termination. At the same time, transcriptional initiation dramatically decreases, due to the RNAPII pool being depleted by ubiquitylation-mediated protein degradation.

We have now investigated the effect on transcription after another ancient cell stress, namely heat-shock. Surprisingly, heat shock results in a dramatic change to transcriptional termination. The results of our analysis of this pathway will be presented.

Jeffrey Chao

Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland

Imaging the life and death of mRNAs in single cells

After transcription, an mRNA's fate is determined by an orchestrated series of events (processing, export, localization, translation and degradation) that is regulated both temporally and spatially within the cell. In order to more completely understand these processes and how they are coupled, it is necessary to be able to observe these events as they occur on single molecules of mRNA in real-time in living cells. To expand the scope of questions that can be addressed by RNA imaging, we are developing multi-color RNA biosensors that allow that status of a single mRNA molecules (e.g. translation or degradation) to be directly visualized and quantified.

In order to image the first round of translation, we have developed TRICK (translating *R*NA imaging by coat protein knock-off) which relies on the detection of two fluorescent signals that are placed within the coding sequence and the 3'UTR. In this approach, an untranslated mRNA is dual labeled and the fluorescent label in the coding sequence is displaced by the ribosome during the first round of translation resulting in translated mRNAs being singly labeled. A conceptually similar approach was used for single-molecule imaging of mRNA decay, where dual-colored mRNAs identify intact transcripts, while a single-colored stabilized decay intermediate marked degraded transcripts (TREAT, 3′ RNA end accumulation during turnover). We are using these tools to characterize localized translation and degradation during normal cell growth and stress.

Keynote

Davide Ruggero

UCSF Helen Diller Family Comprehensive Cancer Center, San Francisco, CA, United States of America

Translating the cancer genome one codon at a time and its therapeutic implications.

Our research is centered on understanding translational control of gene expression in both normal health and disease in a cell- and tissue-specific manner, with a particular focus on cancer biology. Our research combines mouse genetics with genome-wide translational profiling, in-depth molecular biology, and pharmacology to systematically define the points of regulation, in cis and trans, by which the genome is selectively decoded into proteins. We have uncovered that a common denominator of multiple oncogenic pathways is their ability to directly control the core translation machinery of a cell, resulting in the rapid rewiring of mRNA translation programs that promote distinct hallmarks of cancer development such as cell growth, metabolism, and increased motility. For example, our most recent findings delineate the in vivo requirements for a distinct threshold of the major cap-binding protein, eIF4E, in normal organismal development compared to that required for translating the cancer genome. We show that cancer cells require increased eIF4E activity for their survival as distinct subsets of mRNAs that regulate the cancer cell oxidative response are marked by the presence of a novel eIF4E-dependent cis-acting translational control motif present in their 5'UTRs. I will discuss the first screen designed to understand the synthetic lethal interactome of the cancer translatome. Our screen unveiled new functional connections between the major cap-binding protein eIF4E and unexpected cellular processes, including the identification of a novel mitochondria unfolded protein response that surprisingly triggers autophagymediated survival in cancer cells. I will also discuss how translational cues allow for physiological adaptations to nutrient availability in cell-autonomous and non-autonomous manners to maintain metabolic fitness and healthspan in vivo. As nutrient abundance drives anabolic processes, including protein synthesis, we set out to test how eIF4E levels influence metabolic homeostasis linked to diet and the cellular environment. We find that mice with a 50% loss of eIF4E expression show resistance to induced obesity, improved glucose tolerance, and a decrease in hepatic steatosis and cancer induced by obesity. Together, our data reveal that diminished eIF4E levels can promote enhanced metabolic fitness and may be a potential therapeutic target for the treatment of obesity-related disease. The immediate impact of our research has been the design of a new generation of compounds to target the aberrant translation machinery in cancer cells, which are currently in clinical trials, and may reflect a new frontier in cancer therapy.

Dominique Weil

Laboratory of Developmental Biology, Institute of Biology Paris-Seine, Paris, France

The GC content of mRNAs shapes their storage in human P-bodies and their decay

Gene expression depends on the balance between RNA synthesis, decay and translation. In eukaryotes, RNAs and their associated proteins can condensate into cytoplasmic membrane-less organelles called P-bodies. Following the purification of P-bodies from human cells, we could show that they are primarily involved in the coordinated storage of poorly translated mRNAs. These mRNAs encode a large variety of proteins, which tend to have regulatory functions. Combining this dataset with various transcriptomic analysis led us to propose an integrative model of post-transcriptional regulation, where the mRNA GC content plays a key role in coordinating a coherent but nevertheless adaptable gene expression program.

Matthias Altmeyer

University of Zurich, Switzerland

Dealing with DNA lesions across cell cycle boundaries

Genome integrity maintenance is tightly linked to chromatin dynamics and to controlled genome reorganization as cells progress through the cell cycle. In this training unit, I would like to cover recent insights into how dividing cells cope with endogenous DNA lesions that occur at fragile regions of the genome as byproducts of genome duplication. I would like to discuss how such lesions, if they cannot be resolved immediately when they occur, can be dealt with in subsequent cell cycle phases, and even after mitotic cell division, and how this in turn affects genome organization, stability and function.

Monika Gullerova

University of Oxford, United Kingdom

Nascent RNA silencing: novel gene expression regulation pathway

In mammalian cells, small non-coding RNAs (sncRNAs) negatively regulate gene expression in a pathway known as RNA interference (RNAi). RNAi can be categorised into post-transcriptional gene silencing (PTGS), which involves the cleavage of target messenger RNA (mRNA) or inhibition of translation in the cytoplasm, and transcriptional gene silencing (TGS), which is mediated by the establishment of repressive epigenetic marks at target loci. Transfer RNAs (tRNAs), which are essential for translation, can be processed into small ncRNAs, termed tRNA-derived small RNAs (tsRNAs). The biogenesis of tsRNAs and their role in gene expression regulation has not yet been fully understood. Here we show that Dicer dependent tsRNAs promote gene silencing through a mechanism distinct from PTGS and TGS. tsRNAs can lead to downregulation of target genes by targeting introns in via nascent RNA silencing (NRS). Furthermore, we show that Ago2 slicer activity is essential for this nuclear mechanism. Synthetic tsRNAs can significantly reduce expression of a target gene at both RNA and protein levels. Target genes regulated by NRS are associated with various diseases, which further underpins its biological significance. Finally, we show that NRS is evolutionarily conserved and has the potential to be explored as a novel synthetic sRNA based therapeutic.

Fabrizio d'Adda di Fagagna

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IGM-CNR – Istituto di Genetica Molecolare-Consiglio Nazionale delle Ricerche, Pavia, Italy

Non coding RNA synthesis at DNA lesions

Our group has previously reported that DNA double-strand breaks (DSBs) trigger the synthesis by RNA polymerase II of damage-induced long non-coding RNA (dilncRNA) that can be processed into shorter DNA damage response RNAs (DDRNAs). Such transcripts are essential for full DDR activation and their inhibition by antisense oligonucleotides (ASO) allows site-specific inhibition of DNA damage signalling and repair (Francia et al Nature 2012, Michelini et al Nature Cell Biology 2017, D'Alessandro et al Nature Communications 2018).

We recently discovered that such transcriptional events depend on the assembly

of seemingly fully functional transcriptional promoters that include a complete RNA polymerase II preinitiation complex (PIC), MED1 and CDK9. Absence or inactivation of any of these factors causes a reduction in the activation of the DNA damage response (DDR) both in cells and in an in vitro system that reconstitutes DDR activation events on nucleosomes.

Importantly, dilncRNAs drive molecular crowding of DDR proteins, such as 53BP1, into globular structures that exhibit liquid–liquid phase-separation condensate properties (Pessina et al Nature Cell Biology 2019). Transcription at DNA ends is promoted by the MRN complex by melting DNA ends (Sharma et al. Cell Reports 2021).

Telomeres, the ends of linear chromosomes, progressively accumulate DNA damage during physiological and pathological aging. We recapitulated the above-described events at damaged telomeres (Rossiello et al. Nature Communications 2017) and demonstrated that, in independent animal models of accelerated aging, specific DDR inhibition at telomeres by ASO improves aging's detrimental phenotypes and extends lifespan (Aguado et al. Nature Communications 2019).

Aura Carreira

Institut Curie, Orsay, France

BRCA2 promotes DDX5-mediated DNA-RNA hybrid resolution at DNA double-strand breaks to facilitate their repair

DNA-RNA hybrids can form at DNA double-strand breaks (DSBs) in transcribed regions where the nascent transcript hybridizes with the template DNA precluding efficient DSB repair. The tumor suppressor BRCA2 is involved in DSB repair by homologous recombination. BRCA2 deficient cells accumulate DNA-RNA hybrids; however, its possible role at these structures is not well understood. In this talk, I will show our recent findings indicating that BRCA2 and DDX5 cooperate in the resolution of DNA-RNA hybrids associated with DSBs favoring their repair by homologous recombination (HR). I will show how using biochemical assays we could demonstrate that BRCA2 interacts directly with DDX5 and enhances its DNA-RNA unwinding activity by modulating its ATPase activity. In addition, a stable cell line specifically expressing a BRCA2 breast cancer variant that reduces BRCA2-DDX5 interaction exhibit increased frequency of DNA-RNA hybrids and slower kinetics of repair by HR suggesting that these DNA-RNA hybrids represent impediments for DSB repair. Finally, I will present a model on how we think this function may play out in the cell.

Fred Chédin

UC Davis, CA, United States of America

Understanding co-transcriptional R-loop formation and its link to genome instability

R-loop structures are a prevalent class of alternative non-B DNA structures that form during transcription upon invasion of the DNA template by the nascent RNA. R-loops form universally in the genomes of organisms ranging from bacteriophages, bacteria, and yeasts to plants and animals, including mammals. A growing body of work has linked these structures to both physiological and pathological processes, in particular to genome instability.

I will start by reviewing results from R-loop mapping studies and discuss the genomics technologies developed over the last decade to permit R-loop formation. In mammalian genomes, R-loops form over tens of thousands of conserved genic hotspots and collectively cover about 5% of the genomic space. This makes R-loops one of the most abundant non-B DNA structure known to date. The recent development of high-throughput single-molecule R-loop sequencing approaches further reveal that R-loops are giants in the non-B DNA world, covering 350 base-pairs per individual structure on average and often extending to kilobase lengths. Importantly, the availability of R-loop maps has allowed scientists to reveal chromatin signatures enriched over R-loop regions. These signatures will be introduced, and their functional significance discussed.

Understanding the functional outcomes of R-loop formation in genomes requires understanding the forces that drive R-loop formation. I will present an overview of how DNA sequence and DNA topology cooperate to regulate not only the probability of R-loop formation, but also the distribution of R-loops along the DNA fiber, and the stability of the resulting structures. I will explain how these new insights reveal R-loops in a new light as powerful and reversible topological stress relief valves and how this realization expands our view of the potential biological roles R-loops may play under both normal and aberrant conditions.

Finally, I will discuss the links between impaired transcription and co-transcriptional processes, R-loop formation, and genome instability, highlighting gaps in knowledge and possible flaws in our understanding of these relationships.

Gaëlle Legube

Centre de Biologie Intégrative, Toulouse, France

Chromatin and chromosome dynamics at DNA double Strand Breaks

DNA double-strand breaks (DSBs) are highly toxic lesions that are rapidly repaired by two main pathways, namely Homologous Recombination (HR) and Non Homologous End Joining (NHEJ). Using a cell line, called DIvA (for DSB Inducible via AsiSI), where multiples breaks are induced at annotated positions, combined with genome-wide, high throughput sequencing based techniques (ChIP-seq, HiC...) we investigate the contribution of chromatin and chromosome conformation in the response to DSB.

Schragi Schwartz

Weizmann Institute of Science, Rehovot, Israel

Cracking the epitranscriptome

Over 150 types of distinct chemical modifications are catalyzed post-transcriptionally across different classes of RNA. In an analogous manner to well-studied modifications on DNA (e.g. 5-methylcytosine) and on proteins (e.g. phosphorylation), RNA modifications play fundamental roles in regulating the RNA life-cycle and in modulating cellular and physiological responses. The majority of these modifications are evolutionary ancient and highly conserved, and their disruption results in diverse human pathologies. RNA modifications are also of wide interest from biotechnological and therapeutic angles, the latest success being the highly modified SARS-CoV-2 RNA vaccines. RNA modifications were traditionally studied in highly expressed rRNA and tRNA molecules, yet were biochemically intractable in mRNA, for technical reasons. In recent years, genomic approaches developed by us and by others have facilitated the acquisition of unbiased, genome-wide maps of a subset of modifications. I will discuss approaches for detection modifications, challenges associated with them, and highlight advances in our understanding of their distribution, regulation and function. As a case in point I will discuss our recent work, in which we developed an approach for de-novo detection of RNA acetylation sites, allowing us to uncover a thermoadaptive role for dynamic RNA acetylation (Sas-Chen et al, Nature, 2020).

Anne E. Willis

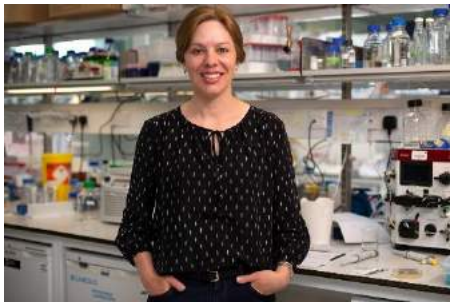
Medical Research Council Toxicology Unit, University of Cambridge, Tennis Court Rd, Cambridge CB2 1QW, United Kingdom

Post-transcriptional of gene expression control following toxic injury and in disease

Eukaryotic cells respond to toxic injury/cell stress by altering gene expression at both the transcriptional and post-transcriptional level and there is overwhelming evidence to demonstrate that post-transcriptional control plays a major role in the cellular stress response, independently of transcriptional control. In the cytoplasm post-transcriptional control is, for the most part, mediated by RNA binding proteins (RBPs), which through their interactions with specific RNA motifs and structural elements regulate all stages of RNA metabolism including its localisation, storage, translation and degradation. Both protein centric and RNA centric methodologies have been developed to assess how post-transcriptional control contributes to gene regulation. For example, to gain an understanding of how the translatome is reprogrammed following cell stress ribosome or polysome profiling can be used and we have carried out these types of analyses following exposure of cells to a variety of cells stresses and also in diseased tissues. We have shown recently that the translatome is reprogrammed following exposure to toxic asbestos fibres, by modulation of translation initiation, and that this is associated with the development of the fatal tumour of the pleura or peritoneum, malignant mesothelioma (MpM). Our data demonstrate that in MpM there is a selective increase in the translation of mRNAs encoding proteins required for ribosome assembly and mitochondria biogenesis. This results in an enhanced rate of mRNA translation, abnormal mitochondrial morphology and oxygen consumption rates, and a reprogramming of metabolic output. In addition, a wide range of high-through-put RBP centric technologies have been developed for assessing changes in RBPs including RNA interactome capture and OOPs, and we also have used these technologies to identify the RBPs whose binding is changed following cell stress. In particular, we have identified an RNA binding protein complex that senses nucleic acid damage in the cytoplasm and have shown mechanistically how this results in cell cycle arrest.

Lori Passmore

MRC Laboratory of Molecular Biology, Cambridge, United Kingdom



Mechanistic insights into the cleavage and polyadenylation machinery

Almost every eukaryotic mRNA has a poly(A) tail, which is added by a 1 MDa multi-protein complex called Cleavage and Polyadenylation Factor (CPF/CPSF). There are four different enzymes within CPF (endonuclease, poly(A) polymerase and two protein phosphatases). CPF is organized into three modules based around these enzymatic activities. We determined a structure of polymerase module subunits Cft1, Pfs2 and Yth1 using cryoEM (Casañal, Kumar, et al., 2017), and a structure of the complex between nuclease module subunits Ysh1 and Mpe1 using X-ray crystallography (Hill, et al., 2019). We have also used biochemical reconstitution, cryoEM and NMR to gain additional insights into the mechanisms of mRNA polyadenylation. We propose that dynamics within CPF are required for efficient and specific polyadenylation, for activation the enzymes within CPF and to co-ordinate transcription and 3′-end processing.

Florence Besse

Institut de Biologie Valrose, Nice, France

Subcellular targeting of mRNAs

Intracellular targeting of mRNAs has recently emerged as a prevalent mechanism used by a variety of polarized and non-polarized cells to compartmentalize protein synthesis. In this lecture, I will describe recent transcriptome-wide studies that led to the systematic identification of mRNAs with specific subcellular distribution. I will also present the nature and regulation of the cellular machineries involved in RNA targeting.

Keynote

Karlene A. Cimprich

Stanford University School of Medicine, CA, United States of America

Department of Chemical and Systems Biology

RNA Meets DNA: Dangerous Liaisons in the Genome

Our genomes are constantly threatened by endogenous and exogenous sources of DNA damage, and a growing body of evidence implicates R-loops as an important endogenous source of genomic instability. R-loops are three-stranded nucleic acid structures consisting of an RNA-DNA hybrid and displaced single-stranded DNA. They are thought to form during transcription when the nascent RNA transcript hybridizes with the DNA template, and various physiological processes are regulated by these structures on chromatin. However, unscheduled or increased levels of R-loops, which can arise when factors that normally regulate their formation are perturbed, can cause the accumulation of DNA damage. In this talk, we will summarize our recent work in this area, focusing on the processes driving R-loop-induced DNA damage and genome instability, and new methods to detect and analyze these events.

Benoit Palancade

Institut Jacques Monod, Université de Paris, France

The impact of genome organization on transcription-dependent genetic instability

Multiple DNA- and RNA-related transactions coexist in eukaryotic nuclei, their synchronization being critical for genome homeostasis. In this frame, transcription and mRNA metabolism have recently emerged as unappreciated players in the maintenance of genome stability. While regulated gene expression is critical for the DNA damage response, high levels of transcriptional activity can also be detrimental for genetic integrity. Among the genotoxic structures that can accumulate upon transcription are the R-loops, which consist of stable hybrids formed between the template strand of the transcribed DNA and the nascent mRNA species, thereby generating a displaced single-stranded DNA. Whether the fate of R-loops depends on the spatial organization of the genome remains however largely unexplored. Using the unicellular eukaryote *S. cerevisiae* as a model, we have investigated how genome structure and nuclear organization impact on R-loop metabolism and transcription-associated genome instability. On the one hand, we uncovered the presence of intronic sequences as one of the major features protecting against R-loop formation in the yeast genome. On the other hand, we found that when R-loops form, they trigger the repositioning of the corresponding loci within the yeast nucleus. The molecular mechanisms ensuring R-loop prevention and resolution in such situations will be discussed.

Chunlong Chen

Institut Curie, Université PSL, Sorbonne Université, Paris, France

The impact of transcription-mediated replication stress on genome instability and human disease

DNA replication is a vital process in all living organisms. At each cell division, > 30,000 replication origins are activated in a coordinated manner to ensure the duplication of > 6 billion base pairs of the human genome. During differentiation and development, this program must adapt to changes in chromatin organization and gene transcription: its deregulation can challenge genome stability, which is a leading cause of many diseases including cancers and neurological disorders. Over the past decade, great progress has been made to better understand the mechanisms of DNA replication regulation and how its deregulation challenges genome integrity and leads to human disease. Growing evidence shows that gene transcription has an essential role in shaping the landscape of genome replication, while it is also a major source of endogenous replication stress inducing genome instability. During the course, I will discuss the current knowledge on the various mechanisms by which gene transcription can impact on DNA replication, leading to genome instability and human disease.

Dipanjan Chowdhury

Harvard Institutes of Medicine, Boston, MA, United States of America

Deciphering end resection at a DNA double strand break

Double stranded DNA break (DSB)s are repaired by two major mechanistically distinct pathways, homologous recombination (HR) and non-homologous end joining (NHEJ). A decisive factor in the choice between DSB repair pathways is in the competition between DNA end protection (necessary for NHEJ) and DNA end resection (necessary for HR). As the 'master regulator' of DSB repair pathway choice, 53BP1, a chromatin-associated reader of epigenetic marks at DSBs, helps channel DSBs into the NHEJ pathway by restricting BRCA1-dependent end resection. 53BP1 nucleates the assembly of a higher order ensemble that includes the Shieldin complex, CST complex, and DNA polymerase (Pol) □-primase complex. Loss of BRCA1 provides a therapeutic opportunity as these tumors are exquisitely sensitive to inhibitors of poly (ADP-ribose) polymerase (PARP), and are also susceptible to platinum-based drugs. Loss of 53BP1 or any of the 53BP1-interacting proteins restores end resection and causes PARPi resistance in BRCA1-mutant tumors. We recently identified Dynein light chain like protein 1 (DYNLL1) as as anti-resection factor. Loss of DYNLL1 allowed DNA end resection and restored HR, thereby inducing resistance to platinum drugs and PARPi in BRCA1-mutant tumors. At the molecular level, DYNLL1 limited the nucleolytic resection of DNA ends by interacting with the resection machinery. Importantly, purified DYNLL1 interacted with MRE11 and inhibited its exonuclease activity in vitro. Now we observe that 53BP1 loss prevents DYNLL1 recruitment to DSBs and more broadly to chromatin. We hypothesize that 53BP1 inhibits MRE11-mediated DNA end resection by recruiting DYNLL1 and that DYNLL1 functions in parallel of Shieldin-CST to regulate DNA end resection and DSB repair pathway choice. We are investigating the crosstalk between the Shieldin-CST and DYNLL1-MRE11 arms of the 53BP1-dependent anti-DNA end resection machinery.

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