# Chromatin organization & Genome stability

Safed, Israel 2023


**Organizers:**

**Itay Onn**

**Yaakov Maman**

**Avi Matityahu**

**Michal Easton Mor**

# The Chromatin Organization & Genome Stability Meeting

September 5-7, 2023

Safed, Israel

## Venue

Ruth Safed Hotel
Tet Zayin St. 1a, Artist Colony
Safed. Israel

## Organizers

Itay Onn
Kobi Maman
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Chromatin Organization & Genome Stability - Safed, Israel, 2023

Outline:

| Tuesday - 5.9 | | |
|---|---|---|
| **Arrival and registration** | | |
| Session I **Chromatin Organization** 10:00-13:30 Chair: Damien D'Amours | **Opening remarks** | |
| | **Amnon Harel** | ***Yeast chromatin landscape: a bird's-eye view*** |
| | **Noam Kaplan** | ***Sensitive high-resolution measurement of promoter-centric interactions corroborates the enhancer-promoter looping model*** |
| | **Joseph Shlomai** | ***The telomere binding protein UMSBP2 is chromatin remodeler that functions in regulation of gene expression and suppression of antigenic variation in trypanosomes*** |
| | Coffee break | |
| | **Orly Avni** | ***Ezh2 harnesses the intranuclear actin cytoskeleton to remodel chromatin in differentiating Th cells*** |
| | **Erez Lieberman Aiden** | ***The Shape of DNA*** |
| Lunch 13:10-14:15 | | |
| Session II **DNA Damage in Chromatin Context** 14:30-18:30 Chair: Meital Gal-Tanamy | **Batsheva Kerem** | ***The Complex Nature of Recurrent Genomic Instability and Its Importance in Cancer*** |
| | **Michael Blank** | ***Exploring Genome Integrity Maintenance and Cancer Molecular Pathways through the Prism of the*** |
| | **Shay Covo** | ***Developmental Regulation of UV-Induced Translation in Fusarium Species*** |
| | **Elnatan Bitensky** | ***Delineating gene expression in response to UV irradiation*** |
| | Coffee break | |
| | **Yaakov Maman** | ***From genotoxicity to oncogenicity: on Helicobacter pylori-induced DNA damage in gastric cells*** |
| | **Diala Shatleh-Rantisi** | ***Studying DNA double strand breaks patterns in cancer cells under replication stress*** |
| | **Yehuda Tzfati** | ***The helicase RTEL1 coordinates telomere length regulation and genome stability: lessons from Hoyeraal-Hreidarsson Syndrome*** |
| | **Riham Smoom** | ***Telomouse – a mouse model with human-length telomeres generated by a single amino acid change in RTEL1*** |
| **Check-in 18:30 - 19:30** | | |
| Dinner party 20:00 - Bat-Ya'ar | | |

| Wednesday - 6.9 | | |
|---|---|---|
| Session III **Chromatin Regulation in Health and Disease (part I)** 8:30-10:00 Chair: Shikha Laloraya | **William Earnshaw** | ***Mitotic chromosome structure and formation*** |
| | **Meir Shamay** | ***KSHV utilizes LINE-1 transposable elements to modulate cellular identity and proliferation*** |
| | **Meital Gal-Tanamy** | ***Epigenetic signatures post Hepatitis C virus cure by direct-acting antivirals*** |
| Tour - 10:30-13:30 | | |
| Lunch 13:30-14:30 | | |
| Session III **Chromatin Regulation in Health and Disease (part II)** 14:30-17:30 Chair: Ludovic Deriano | **Itamar Harel** | ***Exploring the role of interferon signaling as a modifier of genomic instability syndromes*** |
| | **Chaim Cohen** | ***Maintaining healthy longevity by SIRT6 via chromatin*** |
| | **Antonio Musio** | ***Genome instability is a marker of Cornelia de Lange syndrome cells*** |
| | Coffee break | |
| | **Rachel Eiges** | ***Deciphering the role of SMCHD1 in disease and development*** |
| | **Ron Nagar** | ***SIRT6 as Master Regulator of Chromatin Structure in Aging*** |
| | **Itay Onn** | ***Inhibition of SMC complexes' activity by peptides*** |
| Dinner 18:00-19:00 | | |
| Session IV **Chromatin Modifiers** 19:00-20:20 Chair: Shay Covo | **Evan Elliott** | ***Forebrain neuronal Smc3 regulates weight and metabolism partly through Melanocortin 4 receptor*** |
| | **Nabieh Ayoub** | ***Transcriptional Regulation at DNA Double-Strand Break Sites: A Spotlight on Lysine Crotonylation*** |
| | **Shikha Laloraya** | ***A SIR-independent role for Cohesin in subtelomeric silencing and organization*** |
| **Poster session** - 20:20 - 22:00 | | |

| Thursday - 7.9 | | |
|---|---|---|
| Session VI **DNA Damage Response** 9:00-14:00 Chair:Antonio Musio | **Yossi Shiloh** | ***Ataxia-Telangiectasia: A Solid Demonstration of the Genome Stability-Aging-Senescence Link*** |
| | **Feras Machour** | ***Targeting RBM10 deficiency in lung adenocarcinoma*** |
| | **Ludovic Deriano** | ***Alternative repair of DNA double-strand breaks in recombining lymphocytes*** |
| | **Tamar Yulzary-Listovsky** | ***Disrupting translesion synthesis (TLS) activity enhances cell death and prevents tumor growth*** |
| | Coffee break | |
| | **Damien D'Amours** | ***Compaction of chromatin domains by the Smc5/6 complex enhances repair of R-loops*** |
| | **Osama Hidmi** | ***R-loops and Topoisomerase 1 facilitate formation of transcriptional DSBs at gene bodies of hypertranscribed cancer genes*** |
| | **Sara Oster** | ***Unraveling DNA Double-Strand Breaks in Breast Carcinogenesis for Early Detection*** |
| | **Open Discussion- New frontiers in chromatin organization & genome stability** | |
| | Concluding remarks | |
| Lunch and departure - 14:00 | | |

# Program

## Tuesday, September 5

**8:00- Arrival & Registration**

**Session I: Chromatin Organization**
Chair: Damien D'Amours

**10:00 - Opening remarks**
**10:15** - **Amnon Harel,** Bar-Ilan University, Israel
*Yeast chromatin landscape: a bird's-eye view*
**10:40** - **Noam Kaplan**, Technion – Israel Institute of Technology, Israel
*Sensitive high-resolution measurement of promoter-centric interactions corroborates the enhancer-promoter looping model*
**11:05** - **Joseph Shlomai**, The Hebrew University, Israel
*The telomere binding protein UMSBP2 is chromatin remodeler that functions in regulation of gene expression and suppression of antigenic variation in trypanosomes.*

**11:30 – 12:00 Coffee break**

**12:00 - Orly Avni**, Bar - Ilan University, Israel
*Ezh2 harnesses the intranuclear actin cytoskeleton to remodel chromatin in differentiating Th cells*
**12:25 - Erez Lieberman Aiden**, Baylor College of Medicine, USA
*The Shape of DNA*

**13:10 -14:15 Lunch break**

**Session II: DNA Damage in Chromatin Context**

Chair: Meital Gal-Tanamy

**14:30 - Batsheva Kerem**, The Hebrew University, Israel
*The Complex Nature of Recurrent Genomic Instability and Its Importance in Cancer*

**15:10 - Michael Blank**, Bar-Ilan University, Israel
*Exploring Genome Integrity Maintenance and Cancer Molecular Pathways through the Prism of the E3 Ubiquitin Ligase SMURF2*

**15:35 - Shay Covo**, The Hebrew University, Israel
*Developmental Regulation of UV-Induced Translation in Fusarium Species*

**16:00 - Elnatan Bitensky,** The Hebrew University, Israel
*Delineating gene expression in response to UV irradiation*

**16:20 – 16:50 Coffee break**

**16:50 - Yaakov Maman**, Bar-Ilan University, Israel
*From genotoxicity to oncogenicity: on Helicobacter pylori-induced DNA damage in gastric cells*

**17:15 - Diala Shatleh-Rantisi**, The Hebrew University, Israel
*Studying DNA double strand breaks patterns in cancer cells under replication stress*

**17:35 - Yehuda Tzfati,** The Hebrew University, Israel
*The helicase RTEL1 coordinates telomere length regulation and genome stability: lessons from Hoyeraal-Hreidarsson Syndrome*

**18:00 - Riham Smoom**, The Hebrew University, Israel
*Telomouse – a mouse model with human-length telomeres generated by a single amino acid change in RTEL1*

**18:30-19:30 – Check-in**
**20:00 Dinner at Bat-Ya'ar Ranch**

## Wednesday, September 6

**Session III: Chromatin Regulation in Health and Disease (Part 1)**
Chair: Shikha Laloraya

**8:30 - William Earnshaw**, University of Edinburgh, UK
*Mitotic chromosome structure and formation*
**9:10 - Meir Shamay**, Bar-Ilan University, Israel
*KSHV utilizes LINE-1 transposable elements to modulate* cellular identity and proliferation
**9:35 - Meital Gal-Tanamy**, Bar-Ilan University, Israel
*Epigenetic signatures post Hepatitis C virus cure by direct-acting antivirals*

**10:30-13:30 Professional Tour in Safed**

**13:30 -14:30 Lunch break**

**Session III: Chromatin Regulation in Health and Disease (Part 2)**
Chair: Ludovic Deriano

**14:30 - Itamar Harel**, The Hebrew University, Israel
*Exploring the role of interferon signaling as a modifier of genomic instability syndromes*
**14:55 - Haim Cohen**, Bar-Ilan University, Israel
*Maintaining healthy longevity by SIRT6 via chromatin*

**15:20 - Antonio Musio,** ISTI-CNR, Italy
*Genome instability is a marker of Cornelia de Lange syndrome cells*
**15:45 – 16:15 Coffee break**

**16:15 - Rachel Eiges**, The Hebrew University, Israel
*Deciphering the role of SMCHD1 in disease and development*
**16:40 - Ron Nagar,** Bar-Ilan University, Israel
*SIRT6 as Master Regulator of Chromatin Structure in Aging*
**17:05 - Itay Onn**, Bar-Ilan University, Israel
*Inhibition of SMC complexes' activity by peptides*

**18:00-19:00 Dinner**

**Session IV: Chromatin Modifiers**
Chair: Shay Covo

**19:00 - Evan Elliott**, Bar-Ilan University, Israel
*Forebrain neuronal Smc3 regulates weight and metabolism partly through Melanocortin 4 receptor.*
**19:25 - Nabieh Ayoub,** Technion, Institute of Technology, Israel
*Transcriptional Regulation at DNA Double-Strand Break Sites:A Spotlight on Lysine Crotonylation*
**19:50 - Shikha Laloraya**, Indian Institute of Science, Bangalore, India
*A SIR-independent role for Cohesin in subtelomeric silencing and organization*

**<u>Session V:</u> 20:20 - 22:00 Posters, Beer & Wine**

## Thursday, September 7

**Session VI: DNA Damage Response**
Chair: Antonio Musio

**9:00 - Yossi Shiloh**, Tel Aviv University, Israel
*Ataxia-Telangiectasia: A Solid Demonstration of the Genome Stability-Aging-Senescence Link*
**9:40 - Feras Machour,** Technion, Institute of Technology, Israel
*Targeting RBM10 deficiency in lung adenocarcinoma*
**10:05 - Ludovic Deriano,** Institut Pasteur, France
*Alternative repair of DNA double-strand breaks in recombining lymphocytes*
**10:30 - Tamar Yulzary-Listovsky,** Ariel University, Israel
*Disrupting translesion synthesis (TLS) activity enhances cell death and prevents tumor growth*

**10:55– 11:25 Coffee break**

**11:25 - Damien D'Amours**, University of Ottawa, Canada
*Compaction of chromatin domains by the Smc5/6 complex enhances repair of R-loops*
**11:50 - Sara Oster,** The Hebrew University, Israel
*Unraveling DNA Double-Strand Breaks in Breast Carcinogenesis for Early Detection*

**12:10 - Osama Hidmi,** The Hebrew University, Israel
*R-loops and Topoisomerase 1 facilitate formation of transcriptional DSBs at gene bodies of hypertranscribed cancer genes*
**12:30 Open Discussion - New frontiers in chromatin organization & genome stability**
Narrators: Itay Onn and Kobi Maman, Bar-Ilan University, Israel
**13:00 - Concluding remarks**

**13:30 - Lunch and Departure**

# Abstracts

## Tuesday, September 5

## Session I: Chromatin Organization

### Yeast chromatin landscape: a bird's-eye view

Katreena Yamin[1], Boris Fichtman1, Fadia Zagairy[1], Ola Orgil[1], Avi Matityahu[1], Amnon Harel[1] and Itay Onn[1]

1. Azrieli Faculty of Medicine, Bar-Ilan University, Safed, Israel

*Saccharomyces cerevisiae* is an important model organism for studying chromatin structure and associated factors. Although chromosome organization is evolutionarily conserved throughout eukaryotes, two properties distinguish yeast mitotic chromosomes from their mammalian counterparts. Their condensation level from interphase to mitosis is only about 1.5-fold and prometaphase yeast chromosomes cluster into one large mass with a single protruding chromatin loop containing the rDNA locus. We present a new approach for visualizing yeast chromatin by high-resolution scanning electron microscopy (SEM), expanding the available microscopic toolbox for studying chromatin in this organism. Traditional chromosome spreading was modified to avoid air drying and replace with critical point drying and iridium coating. SEM images revealed a three-dimensional landscape with an irregular folding pattern and detailed ultrastructure. We used direct surface imaging of yeast chromatin by SEM to analyze changes in chromatin ultrastructure during the cell cycle and following manipulation of SMC complex activity. Overexpression of histone H2B in mammalian cells induces chromosome individualization in mitosis. Intriguingly, we find that overexpression of histone H2B in yeast cells causes dramatic morphological changes in chromatin structure, as visualized by SEM. These changes are associated with a global misregulation of transcription, but this does not affect genome integrity. Our findings provide new experimental means for studying changes in yeast chromatin landscape and their consequences.

**Sensitive high-resolution measurement of promoter-centric interactions corroborates the enhancer-promoter looping model**

Noam Kaplan[1]

1.Department of Physiology, Biophysics & Systems Biology, Rappaport Faculty of Medicine, Institute of Technology Technion, Israel

The enhancer-promoter looping model, in which enhancers activate their target genes via physical contact, has long dominated the field of gene regulation. However, the ubiquity of this model has been questioned due to evidence of alternative mechanisms and the lack of its systematic validation, primarily owing to the absence of suitable experimental techniques. We present a new MNase-based proximity ligation method called MChIP-C, allowing for the measurement of protein-mediated chromatin interactions at single-nucleosome resolution on a genome-wide scale. By applying MChIP-C to study H3K4me3 promoter-centered interactions in K562 cells, we found that it had greatly improved resolution and sensitivity compared to restriction endonuclease-based C-methods. This allowed us to identify EP300 histone acetyltransferase and the SWI/SNF remodeling complex as potential candidates for establishing and/or maintaining enhancer-promoter interactions. Finally, leveraging data from published CRISPRi screens, we found that most functionally-verified enhancers do physically interact with their cognate promoters, supporting the enhancer-promoter looping model.

**The telomere binding protein UMSBP2 is chromatin remodeler that functions in regulation of gene expression and suppression of antigenic variation in trypanosomes**

Soni Awakash[1], Klebanov-Akopyan Olga[1], Mishra Amartya[1], Glousker Galina[2], Erben Esteban[3], Plaschkes Inbar[4], Benyamini Hadar[4], Mitesser Vera[1], Harel Amnon[5], Yamin Katreena[5], Onn Itay[5], Tzfati Yehuda[2] and Shlomai Joseph[1]

1. Department of Microbiology and Molecular Genetics, The Hebrew University of Jerusalem, Israel
2. Department of Genetics, The Hebrew University of Jerusalem, Jerusalem, Israel.
3. Heidelberg University Center for Molecular Biology, Heidelberg, Germany.
4. The Info-Core Bioinformatics Unit, Faculty of Medicine, The Hebrew University of Jerusalem, Jerusalem, Israel. 5. Azrieli Faculty of Medicine, Bar-Ilan University, Safed, Israel

Universal minicircle sequence binding proteins (UMSBPs) are CCHC-type zinc-finger proteins that bind a single-stranded G-rich DNA sequence (UMS) conserved at the replication origins of the mitochondrial genome (kinetoplast DNA, kDNA) of trypanosomes. Both mitochondrial UMSBP1 and nuclear UMSBP2 proteins function in kDNA replication and segregation. However, only the nuclear protein UMSBP2 plays an essential role in three major DNA transactions in the nucleus: (1) It colocalizes with telomeres at the nuclear periphery, where it interacts with the 3'-single-stranded G-rich DNA strand and protects the chromosomes ends; (2) It is involved in the positioning of telomeres, at the nuclear periphery; (3) It functions in chromatin remodeling, affecting chromatin structure and transcription regulation in the trypanosome cell. *TbUMSBP2* silencing results in impaired nuclear mitosis, telomere clustering in the nucleoplasm, induction of DNA damage response (DDR) at telomeres and impaired nuclear mitosis. Furthermore, UMSBP2 silencing results in a significant decrease in the disassembly of nucleosomes, a phenotype that could be reversed, by supplementing the *UMSBP2* knockdown cells with UMSBP2. Transcriptome analysis revealed that silencing of UMSBP2 affects the expression of multiple genes in the trypanosome cells, with a most significant effect on the upregulation of the subtelomeric *Variant Surface Glycoproteins* (*VSG*) genes, which mediate the antigenic variation in African trypanosomes. Functional homolog of UMSBP2 in higher eukaryotic organisms will be discussed.

**Ezh2 harnesses the intranuclear actin cytoskeleton to remodel chromatin in differentiating Th cells**

Moran Titelbaum[1], Boris Brant[1], Daniel Baumel[1], Alina Burstein-Willensky[1], Shira Perez[1], Yiftah Barsheshet[1], and Orly Avni[1].

1. Azrieli Faculty of Medicine, Bar-Ilan University, Safed, Israel

Following their first interaction with the antigen, quiescent naïve T-helper (Th; $CD4^+$) cells enlarge, differentiate, and proliferate; these processes are accompanied by substantial epigenetic alterations. We found that during Th cell differentiation, the methyltransferase activity of the PcG protein Ezh2 regulates post-transcriptionally inducible assembly of intranuclear actin filaments. These filaments are colocalized with the actin regulators Vav1 and WASp and intermingle with the chromatin fibres. The inducible assembly of nuclear actin filaments is required for chromatin spreading and nuclear growth. Altogether our findings delineate a model in which the epigenetic machinery orchestrates the dynamic mechanical force of the intranuclear cytoskeleton to reorganize chromatin during differentiation.

## Session II: DNA Damage in Chromatin Context

### The complex nature of recurrent genomic instability and its importance in cancer

Batsheva Kerem[1]

1. Department of Genetics, The Life Sciences Institute, The Hebrew University, Jerusalem, Israel

Genomic instability is a hallmark of cancer and a driver of tumorigenesis. Aberrant activation of oncogenes and tumor suppressor genes induces replication stress, leading to accumulation of DNA damage and an increased tumorigenicity potential. Previous studies have shown recurrent instability regions in cancer, which is attributed to positive selection and/or the sensitivity of specific genomic regions to breakage. Among these regions are fragile sites (FSs), genomic regions sensitive to replication stress conditions. Instability at fragile sites is commonly induced by the DNA polymerase inhibitor aphidicolin. We previously have shown that aberrant oncogene expression induces replication stress, leading to DNA breaks and genomic instability. Mapping the cytogenetic locations of oncogene-induced fragility showed that in the same cell type, each oncogene creates a unique fragility landscape that only partially overlaps with aphidicolin-induced FSs. We further explored the molecular mechanism underlying the aphidicolin-induced fragility by analyzing the transcriptional profile and DNA replication timing (RT) under aphidicolin treatment in the context of the 3D genome organization. The results revealed a fragility signature, comprised of a topologically associated domain (TAD) boundary overlapping a highly transcribed large gene with aphidicolin-induced RT-delay. These results identified the role of the genome architecture in maintaining genome stability. More recently, we initiated experiments to explore the molecular basis of oncogene recurrent fragility. We found an aberrant accumulation of R-loops in mutated RAS cells. Furthermore, we revealed a mutated RAS fragility signature, comprising of RT delay at actively transcribed large genes due to aberrant accumulation of R-loops. These results shed a new light on the effect of RAS-induced transcription changes leading to genome stability. The observed plasticity in the fragility landscape of the same cell type following different replication stress inducers highlights an additional level of complexity in the molecular basis for recurrent fragility in cancer.

**Exploring Genome Integrity Maintenance and Cancer Molecular Pathways through the Prism of the E3 Ubiquitin Ligase SMURF2**

Michael Blank[1]

1. Laboratory of Molecular & Cellular Cancer Biology, The Azrieli Faculty of Medicine, Bar- Ilan University

The HECT-type E3 ubiquitin ligase SMURF2 recently emerged as a core regulator of diverse molecular and cellular processes, pertinent to normal cell physiology and pathobiology, especially to cancer. Our investigation focuses on these processes. We discovered that SMURF2 acts as a potent tumor suppressor and epigenetic regulator. The depletion of SMURF2 exerts a profound impact on the ability of the affected cells and tissues to maintain the epigenetic structure landscape and chromosomal architecture, adequately respond to cellular stress, repair DNA damage, and protect genome integrity and cell identity. Mechanistically, we found that SMURF2 regulates the stability and/or activity of several core epigenetic regulators, including histone protein ligase RNF20 and methyltransferase EZH2, DNA topology regulator Topo IIα, poly(ADP-ribose) polymerase PARP1, transcriptional co-repressor and chromatin modifier TRIM28/KAP1, as well as the epi-transcriptome regulator RNA editase ADAR1p110. Moreover, SMURF2 also plays a role in regulating the autophagic-lysosomal turnover of the nuclear structure protein lamin-A and its mutant form progerin, whose expression underlies the development of the premature ageing syndrome HGPS and is associated with physiological ageing and cancer. Our investigations further revealed that SMURF2 expression is significantly altered in malignant tissues and have suggested a link between aberrant SMURF2 expression and tumor aggressiveness. Altogether, these findings point to SMURF2 as a critical factor in regulating key molecular and cellular processes pertinent to malignant transformation and carcinogenesis.

## Developmental Regulation of UV-Induced Translation in Fusarium Species

Shay Covo[1]

1. Faculty of Agriculture, Food and Environment, The Hebrew University, Israel

Ribosome biogenesis is the hallmark of fast growth. Exposure to DNA damage causes cell cycle arrest and consequently reduction in ribosome biogenesis. We observed this phenomenon also in Fusarium species that were exposed to methyl methanesulfonate right after they entered the cell cycle. Interestingly, when we exposed the same species to UV at a later developmental stage, we observed the opposite. Ribosome biogenesis was induced; ribosome biogenesis genes were induced at mRNA level, ribosome themselves and polysomes were accumulated and translation capacity was induced. Remarkably, the UV-induced ribosome biogenesis appeared to operate independently of TOR signaling. Furthermore, inhibiting rRNA synthesis not only blocked the UV-induced ribosome biogenesis but also increased the fungi's susceptibility to UV radiation and reduced UV damage repair. Analyzing the genes that were differentially associated with polysomes after UV exposure revealed modules that belong to proteome health, such as transcription, translation and protein folding. In conclusion, we describe, for the first time, a DNA damage induction of global gene expression machinery. We hypothesize that UV-induced translation counteracts the inhibition imposed by UV on transcription. We further hypothesize that similar pathways are operated in other eukaryotes and therefore, might be used as a target for synthetic lethality with DNA damage.

**Delineating gene expression in response to UV irradiation**

Elnatan Bitensky[1], Elisheva Heilbrun[1], Avital Parnas[1] and Sheera Adar[1]

1. Department of Microbiology and Molecular Genetics, Institute for Medical Research Israel Canada, Faculty of Medicine, Hebrew University of Jerusalem, Ein Kerem, Jerusalem, 91120, Israel.

The exposure of cells to harmful ultraviolet (UV) radiation leads to a multi-pronged response including a genome wide transcriptional shutdown. However, the upregulation of repair genes has been reported in response to UV-induced DNA damage. How these two seemingly competing mechanisms of transcriptional shutdown and genic upregulation coincide remains unknown. One possible explanation to resolve this discrepancy is that these genes are somehow exempt from the genome-wide transcriptional shutdown and can undergo increased transcription. An alternative explanation is that although these genes are indeed transcriptionally repressed along with the rest of the genome, their mRNA transcripts are inherently stable, or undergo stabilization in response to DNA damage. Thus, in comparison with less stable transcripts, these genes appear relatively abundant within the cell. To address this question, we measured transcript stability by exposing cells in culture to DRB (Benzimidazole), an RNA polymerase elongation inhibitor. Without the interference of newly synthesized mRNA transcripts, the measurement of decay and degradation of mRNA transcripts becomes possible. Using this approach, we can systematically observe whether RNA transcripts become stabilized in response to UV irradiation.

**From genotoxicity to oncogenicity: on Helicobacter pylori-induced DNA damage in gastric cells**

Hadas Sibony-Benyamini[1], Rose Jbara[1], Tania Shubash[1], Alexander Brandis[2], Layan Abu-Rahmun[1], Tamar Leshem[3], Avi Peretz[1,3], Yaakov Maman[1]

1. Azrieli Faculty of Medicine in the Galilee, Bar-Ilan University, Zefat, Israel.
2. Life Sciences Core Facilities, Weizmann Institute.
3. Baruch Padeh Medical Center, Poriya, Israel.

*Helicobacter pylori (H.pylori)* infection is a significant risk factor for developing gastric cancer (GC). A growing body of evidence outlines a causal link between infection with *H.pylori* and increased DNA damage in host cells. This association is primarily attributed to the CagA virulence factor which downregulate DNA damage response. In addition, *H.pylori* has been shown to induce DNA damage independently of CagA, but the specific mechanism responsible for this form of genotoxicity is unclear. Additionally, the potential link between the formation of DNA damage upon infection and the emergence of cancer-driving structural variants (SV) remained unexplored.
To bridge these knowledge gaps, we generated a high-resolution genome-wide map of sites harboring *H.pylori* infection-induced double stranded breaks (DSBs).
These *H.pylori*-mediated DSBs were dependent on DNA replications and localized at predetermined replication-related fragile sites. Consistent with that, we found that *H.pylori* infection, independently of CagA, inflicts nucleotide depletion through the disturbance of Rb/E2F1 pathway, and the downregulation of RRM2 expression. Finally, we show that sites of recurrent *H.pylori* mediated breaks coincide with chromosomal deletions observed in patients with intestinal-type GC, and that this link potentially elucidates the persistent transcriptional alterations observed in cancer driver genes

**Studying DNA double strand breaks patterns in cancer cells under replication stress**

Diala Shatleh-Rantisi[1], Rami I. Aqeilan[1]

1. The Lautenberg Center for Immunology and Cancer Research - IMRIC, Faculty of Medicine, Hebrew University of Jerusalem, Jerusalem, Israel

DNA replication and cell division contribute to continuity. These two essential cellular processes are finely regulated by various key proteins and check point pathways during the cell cycle. Before cell division, it is necessary to have complete genome replication. However, DNA replication disturbances could occur leading to DNA replication stress (RS). RS is defined by the reduction of replication fidelity due to altered replication fork progression. RS can occur spontaneously, as mutations accumulate in certain genomic regions, or induced chemically by using drugs that target known RS genes or pathways. RS is sensed by the DNA damage response machinery which activates a myriad of DNA repair proteins and enzymes to solve any DNA damage. If remains unsolved, DNA mutations could accumulate and persist leading to DNA double strand breaks (DSBs) and genomic instability. It is well known that genomic instability is a key driver in cancer. High resolution mapping of RS induced genomic DNA DSBs can help to identify RS vulnerable genomic regions. Furthermore, Understanding the nature of these affected genomic regions could lead to a profound understanding of their susceptibility to breakage and their role in enhancing carcinogenesis. Thus, we aim to study the pattern (distribution) of DNA DSBs in cancer cells, under spontaneous or induced RS conditions. Consequently, we can identify and characterize genomic regions or genes that are prone to DNA DSBs under RS and explore their role in enhancing cancer development.

**The helicase RTEL1 coordinates telomere length regulation and genome stability: lessons from Hoyeraal-Hreidarsson syndrome**

Aya Awad[1], Riham Smoom[1], Noa Hourvitz[1], Hosniyah El Ayoubi[1], Yehuda Tzfati[1]

1. Department of Genetics, The Silberman Institute for Life Sciences, The Hebrew University of Jerusalem, Jerusalem, Israel.

Hoyeraal-Hreidarsson syndrome (HHS), a severe telomere biology disease (TBD), is characterized by accelerated telomere shortening and diverse symptoms including bone marrow failure, immunodeficiency, inflammatory bowel disease, neurodevelopmental defects, and death at early childhood. HHS is caused by germline mutations in telomerase subunits or accessory factors, and in the helicase regulator of telomere elongation 1 (RTEL1). Heterozygous mutations in RTEL1 are associated with cancer, pulmonary fibrosis and other clinical conditions. RTEL1 was reported to interact with multiple proteins and various RNA and DNA structures, and play diverse roles in telomeric, as well as non-telomeric genome stability. However, the mechanisms by which RTEL1 regulates telomere length and genome stability, and how RTEL1 mutations cause severe diseases are not fully understood. To separate specific functions of RTEL1 and study how impairments in these functions contribute to disease manifestations, we established a collection of fibroblasts from HHS patients carrying various point mutations in different domains of RTEL1, and rescued their growth by ectopic expression of the telomerase reverse transcriptase (TERT) subunit and wild-type RTEL1. Using an inducible promoter to turn the ectopic RTEL1 expression on and off revealed direct and indirect consequences of RTEL1 dysfunction. Our results indicate that RTEL1 is critical for both telomeric and genome-wide stability, as well as for the proper localization of various non-coding RNAs. While the main cause of HHS is impaired telomere length maintenance, other compromised RTEL1 functions may contribute to diverse pathological conditions. These results suggest that RTEL1 is a central player in coordinating telomere maintenance, genome stability and non-coding RNA biogenesis. Understanding the mechanisms of RTEL1 functions would enable developing specific therapies for TBD, aging associated diseases and cancer.

**Telomouse – a mouse model with human-length telomeres generated by a single amino acid change in RTEL1**

Riham Smoom[1], Catherine Lee May[2], Mark Tigue[2], Benjamin Kahn[2], Hannah M. Kolev[2], Ashleigh Morgan[2], Nachshon Egyes[1], Dan Lichtental[1], Klaus H. Kaestner[2] and Yehuda Tzfati[1]

1. Department of Genetics, The Hebrew University of Jerusalem.
2. Perelman School of Medicine, University of Pennsylvania, Philadelphia.

Telomeres are the protective structures at the ends of eukaryotic chromosomes. The house mouse, *Mus musculus,* has extremely long telomeres, which hinders its use as a model for studying the role of telomeres in human aging and cancer. The large difference in telomere length between *M. musculus* and *M. spretus* was previously exploited to identify the helicase RTEL1 as a dominant regulator of telomere length. However, the difference in RTEL1 between the two species has not been found. We identified germline mutations in human RTEL1, which cause Hoyeraal-Hreidarsson syndrome (HHS). One of the mutated amino acids in HHS, methionine 492 (M492), is conserved across vertebrates except for a lysine in *M. spretus,* suggesting that this change is responsible for the shorter *spretus* telomeres. To test this hypothesis, we generated a *M. musculus* strain in which M492 was changed to a lysine by CRISPR/Cas9 genome editing in zygotes. The *Rtel1*$^{M492K}$ mice appear healthy, but their telomere length set point gradually shortened to a third of the WT length – the range of human telomeres – over fourteen generations. Importantly, the short mouse telomeres appeared to maintain their structure and protective function, as indicated by their normal telomeric G-rich overhang length, and no increase in DNA damage at telomeres or in telomeric aberrations. The finding that a single amino acid change is responsible for the telomere length difference between the two species provides an important insight into the mechanism of telomere length regulation. Furthermore, the healthy *Rtel1*$^{M492K}$ mouse with short telomeres represents a novel and invaluable model for studying the implications of short telomeres in aging and cancer.

1. Ding, H.*, et al. Cell* 117, 873-886 (2004).
2. Deng, Z.*, et al. PNAS* 110, E3408-3416 (2013).
3. Smoom R, et al. BioRxiv 2021.06.06.447246 (2023).

# Wednesday, September 6

## Session III: Chromatin Regulation in Health and Disease (Part 1)

### Mitotic chromosome structure and formation

Kumiko Samejima[1], Johan Gibcus[2], Sameer Abraham[3], Fernanda Cisneros-Soberanis[1], Itaru Samejima[1], Job Dekker[2], Leonid Mirny[3] and Anton Goloborodko[4], William C. Earnshaw[1]

1. Wellcome Centre for Cell Biology, University of Edinburgh, Scotland, UK
2. University of Massachusetts.
3. Massachusetts Institute of Technology.
4. IMBA, Vienna.

When vertebrate cells divide, the cell nucleus is disassembled, and the genome is packaged into condensed chromosomes with the DNA shortened by a remarkable 10,000-fold. Mitotic chromosomes were first described in the 1880s, and amazingly, still today we do not know how the DNA/chromatin fiber is organised to give this incredibly efficient compaction. My lecture will describe results of an international collaboration using cell biology, next generation genomics and polymer physics that has begun to yield insights into how mitotic chromosomes are organised by the protein complexes cohesin and condensin. For these studies, we have developed a chemical genetic system using an CDK1 analogue sensitive mutant combined with auxin inducible degron cell lines that allows us to analyse highly-synchronised cells at $G_2$ phase and during unprecedented synchronous mitotic entry either in the presence or absence of key proteins. This study began with the surprising discovery that mitotic chromosomes lacking cohesin have a much more ordered 3d architecture than their wild-type counterparts. This led us to rigorously re-examine the levels of cohesin and condensin on prometaphase chromosomes, yielding another surprising result: cohesin is about as abundant on those chromosomes as condensin is (it had previously been thought to leave the chromosome arms during prophase). By combining chemical genetic and auxin knock-down technologies, we could demonstrate that it is cohesive cohesin (holding sister chromatids together) and not loop extruding cohesin (responsible for TAD formation) that influences mitotic chromosome architecture. Molecular simulations revealed the remarkably different outcomes that result when condensin collides with extrusive or cohesive cohesin. Furthermore, because of the extraordinary mitotic synchrony in this system we can for the first time report the speed of loop extrusion by condensin I and II in vivo. I will close by

describing the powerful new polymer models that we have created to describe the organisation of loops within these chromosomes.

**KSHV utilizes LINE-1 transposable elements to modulate cellular identity and proliferation**

Boris Rapoport[1], Anuj Ahuja[1], Supriya Bhattacharya[1], Vyacheslav Gurevich[1], Meir Shamay[1]

1. Daniella Lee Casper Laboratory in Viral Oncology, Azrieli Faculty of Medicine, Bar-Ilan University, Safed, Israel.

Kaposi's sarcoma associated herpesvirus (KSHV, HHV-8) is the causative agent of all forms of Kaposi's sarcoma (KS) and is tightly associated with primary effusion lymphoma (PEL), and multicentric Castleman's disease. Latently infected cells, including KSHV-associated PEL cells, express many dysregulated cellular genes and miRNAs. The Long Interspersed Nuclear Element -1 (LINE-1, L1) is the most abundant transposable element, occupying 17% of the human genome. Since it is still able to transpose, L1 expression is tightly controlled, and generally repressed in somatic cells. Up-regulation of L1 has been detected in several cancers. We have found DNA hypo-methylation, open chromatin, high L1 expression and high RT-activity in PEL cells. Up-regulation of L1 is detected also in de-novo infected B and epithelial cells. To determine the contribution of L1 RT for gene expression observed in KSHV-infected cells, we performed global analysis of miRNA (miRNA-seq) and mRNA (RNA-seq) in cells treated with L1 RT inhibitor. We identified many genes that their high expression in PEL is dependent on L1 RT, including many neuronal genes that should not be expressed in B-cells. Our study sheds a new light on the contribution of L1 on the lose of cell identity, via up-regulation of neuronal genes and down-regulation of B-cell/lymphocyte specific genes. Interestingly, inhibition of L1 RT-activity suppressed PEL cell growth. This observation is relevant for the treatment of KSHV-associated malignancies since they often develop in AIDS patients that are treated with HIV RT inhibitors. Some of these HIV RT inhibitors are also potent inhibitors for L1 RT activity at the appropriate concentration.

## Epigenetic signatures post Hepatitis C virus cure by direct-acting antivirals

Meital Gal-Tanamy[1]

1. Molecular Virology Lab, Azrieli Faculty of Medicine, Bar-Ilan University, Israel

Hepatitis C virus (HCV) is a major cause of death and morbidity globally and the leading cause of hepatocellular carcinoma (HCC). Although now, with new direct-acting antivirals (DAAs) therapy available, HCV is a curable cancer-associated infectious agent, HCC prevalence is expected to continue to rise because HCC risk still persists after HCV cure. Understanding the factors that lead from HCV infection to HCC pre- and post-cure may open-up opportunities to novel strategies for HCC prevention. We recently reported the induction of alterations in the transcriptome of host cells via epigenetic dysregulation by HCV that persist after cure by DAAs as an epigenetic signature. This epigenetic signature is associated with hepatocarcinogenesis. We demonstrate that different treatment regimes show range of persistence of the epigenetic signature that correlate with treatment efficiency. Moreover, we show that the HCV that is a cytoplasmic virus, induce the epigenetic and oncogenic signatures by perturbation of host signaling pathway in the cytoplasm, such as EGFR pathway. We also identified correlation between HCV-induced changes in epigenetic marks associated with chromatin decompaction and mutation loads in HCV-related HCC. Inhibitors for epigenetic modifiers showed promising results as means for reversion of HCV-related epigenetic signature and oncogenic phenotypes. These studies have important contribution for advancing the understanding of the mechanisms of HCV-induced cancer pre and post cure.

**Session III: Chromatin Regulation in Health and Disease (Part 2)**

## Exploring the role of interferon signaling as a modifier of genomic instability syndromes

M. Bergman[1], U. Goshtchevsky[1], T. Atlan[1], G. Astre[1] and I. Harel[1]

1. Department of Genetics, The Silverman Institute, The Hebrew University of Jerusalem

Genomic instability is a key hallmark of aging and related diseases, leading to inflammation, senescence, apoptosis, and cancer. However, strategies to mitigate these degenerative phenotypes are largely lacking. Here, we utilize the naturally short-lived African turquoise killifish to model Ataxia Telangiectasia (ATM) and the Bloom Syndrome (BLM), two human genomic instability syndromes. These models faithfully replicate human disease phenotypes, including infertility, short stature, reduced lifespan, and increased micronuclei. Transcriptomic analysis reveals upregulation of an inflammatory response, particularly interferon signaling. To investigate the link between genomic instability and inflammation, we introduce a mutation to the cytosolic DNA-sensing receptor cyclic GMP–AMP synthase (*cGAS*), demonstrating attenuated cGAMP production following DNA damage. As a strategy to counter age-related phenotypes in genomic instability syndromes, we have generated *ATM;cGAS* double mutants, and are now characterizing their physiological phenotypes. This work provides insights into the interplay between genomic instability and interferon signaling, opening avenues for potential therapeutic interventions.

**Maintaining healthy longevity by SIRT6 via chromatin**

H.Y. Cohen[1,2]

1. Faculty of Life Sciences , Bar-Ilan University, Israel
2. Sagol Healthy Longevity Center Bar-Ilan University, Israel

Sirtuins are NAD+ dependent deacylases, homologues of the yeast SIR2 protein that were shown to play a major role in regulating lifespan and healthspan. Mice over expressing SIRT6, one of the seven mammalian sirtuins, have extended lifespan along with significant improvement of their healthspan. In comparison to their wild-type (WT) littermates, old SIRT6 transgenic (Tg) mice showed amelioration of a variety of age-related disorders, including: improved glucose tolerance, younger hormonal profile, reduced age-related adipose inflammation, increased physical activity and reduced frailty. To explore the mechanisms underlying SIRT6 positive effects on healthy longevity the SIRT6ome, i.e. the SIRT6 dependent metabolome, transcriptome and chromatin profiling were characterized in WT and SIRT6 Tg mice. These analyses demonstrated that SIRT6 overexpression rewired the metabolism of the old animal to a young-liked signaling network. Mechanistically, SIRT6 overexpression mimics key features of the metabolic profile of dietary restriction (DR), a well-known treatment that extends healthy lifespan in multiple organisms. At the chromatin level, SIRT6 control the chromatin structure of specific loci to control its positive effects. Altogether, these findings suggest a new mechanism for the regulation of healthy longevity by SIRT6.

**Genome instability is a marker of Cornelia de Lange syndrome cells**

Maddalena Di Nardo[1], Maria Michela Pallotta[1], Ian D. Krantz[2,3] and Antonio Musio[1]

1. Institute for Biomedical Technologies, National Research Council, Pisa, Italy
2. Roberts Individualized Medical Genetics Center, Division of Human Genetics, The Children's Hospital of Philadelphia, Philadelphia, USA
3. Perelman School of Medicine, The University of Pennsylvania, Philadelphia, USA

Cornelia de Lange syndrome (CdLS) is a rare developmental disorder with an incidence of between 1:10,000 and 1:30,000 live births. Common characteristics of CdLS include cognitive impairment, pre- and postnatal growth retardation, microcephaly, facial dysmorphia, hirsutism, and upper extremity defects. CdLS is caused by mutations in *HDAC8*, *NIPBL*, *RAD21*, *SMC1A* and *SMC3* genes belonging to the cohesin-core or its regulators. Recently, we showed that two CdLS patients carrying a mutation in *SMC1A* gene are characterized by reduced cell life span, high level of oxidative stress and genome instability. Up until now, no systematic study has been performed to investigate whether genome instability is a marker of CdLS patients. To gain insight into this topic, we cultured CdLS cell lines harboring mutations in *SMC3*, *NIPBL* and *HDAC8* genes. We found that CdLS cells became senescent around the 25$^{th}$ passage with a considerable decrease in their in vitro lifespan compared with control cell lines. This senescence was confirmed with a -galactosidase assay. Next, we analyzed the level of oxidative stress during cell progression through in vitro culture. To study global oxidative stress, we measured the level of protein carbonyls by ELISA. At early passage, the protein carbonyl content in CdLS cells was significantly higher than control cells. In addition, the frequency of spontaneous chromosome aberrations was also found to be significantly higher in all-mutated cell lines. These results indicate that genome instability may be considered a specific marker of CdLS.

## Deciphering the role of SMCHD1 in disease and development

Uria Aviel[1,2], Silvina Epsztejn-Litman[1], Merav Hecht[2], Yotam Drier[2] and Rachel Eiges[1,2]

1. Stem Cell Research Laboratory, Shaare Zedek Medical Center, Jerusalem 91031, Israel.
2. The Hebrew University School of Medicine, Jerusalem 91120, Israel.

SMCHD1 modifies chromatin and affects DNA methylation during early embryo development, playing a role in X inactivation and gene cluster silencing. It regulates de novo DNA methylation and chromatin interactions, most likely by antagonizing CTCF binding. Mutations in SMCHD1 can cause two dominant diseases: FSHD2, which leads to DNA hypomethylation and chromatin relaxation, and BAM syndrome, which results in midline defects. It's unclear how SMCHD1 mutations lead to different conditions, and how it mediates chromatin interactions.

The goal of our research is to explore the potential involvement of SMCHD1 in mediating de novo methylation at repetitive elements that are associated with various pathologies other than FSHD. Thus far, we established a dozen SMCHD1 KO hESC clones by gene editing on the background of wild type versus mutant hypermethylated alleles underlying fragile X (CGG expansion in *FMR1*) and myotonic dystrophy type 1 (CTG expansion in *DMPK*) and monitored for a change in DNA methylation. Using bisulfite DNA colony sequencing, we find no evidence for a change in abnormal methylation at either loci. Strikingly though, methylation levels remained unchanged also at the D4Z4 repeats. Nevertheless, we identified several clusters of CpG islands that consistently become hypomethylated. Some correspond with ectopic CTCF binding, supporting the view that SMCHD1 antagonizes CTCF occupancy by hypermethylation in hESCs. Altogether, we conclude that the activity of SMCHD1 in de novo methylation at long repetitive elements are most likely limited to a critical developmental window before blastocyst formation or takes place only following cell differentiation.

**SIRT6 as Master Regulator of Chromatin Structure in Aging**

Ron Nagar[1]

1. Faculty of Life Sciences , Bar-Ilan University, Israel

Aging leads to a gradual decline in physical activity and a disruption of energy homeostasis. Sirtuin 6 (SIRT6), a NAD+-dependent deacylase and mono-ADP ribosyl transferase (mADPr), is intricately involved in numerous cellular pathways that together are implicated in promoting longevity. Although there is a substantial body of data exploring the role of SIRT6 in the chromatin structure of specific promoters, our understanding of its involvement in chromatin dynamics during the aging process remains limited.

To explore the role of SIRT6 in maintaining chromatin homeostasis, a comprehensive approach combining various omics analyses and cell biology techniques was undertaken. We found that SIRT6 plays a critical role in regulating the levels of Lamin A/C, a well-established component of the nuclear lamina. Additionally, SIRT6 influences the levels and localization of H3K9me3, a modification linked to the maintenance of heterochromatin, particularly in the context of mice livers. These findings collectively point towards a novel role for SIRT6 in the maintenance of chromatin integrity associated with the aging process.

**Inhibiting SMC complexes by peptides**

Maria Elias[1], Samar Gani[1], Yana Lerner[1], Katreen Yamin[1], Chen Tor[1], Adarsh Patel[1], Avi Matityahu[1], Moshe Dessau[1], Nir Qvit[1] and Itay Onn[1]

1. The Azrieli Faculty of Medicine, Bar-Ilan University, Safed, Israel

The structural maintenance of chromosome (SMC) complexes, which mediates the three-dimensional structure of chromatin and is involved in maintaining genome stability and function. At the heart of the SMC complexes are two elongated-shaped SMC proteins that dimerize through globular domains at their edges, called head and hinge. The head harbors two half ATPase domains. ATP binding to the SMC heads induces their dimerization and the formation of two active sites, while hydrolysis results in heads disengagement. This ATPase cycle is essential for driving the activity. We report on the development of a peptide to inhibit cohesin, an SMC complex that organizes interphase chromatin and mediates sister chromatid cohesion. The cohesin-inhibiting peptide (CIP) binds Smc3 in vitro with differential binding affinities to the ATP-unbound and bound forms of the protein and inhibits the ATPase activity. Treating yeast cells with the CIP prevents cohesin's tethering activity and, interestingly, leads to the accumulation of cohesin on chromatin. Human cells treated with the peptide show a mototic delay. We developed a second peptide, targeting the corresponding domain in the SMC complex condensin, an SMC complex involved in mitotic condensation. Yeast cells treated with the peptide have a severe condensation defect. Altogether, we demonstrate the power of peptides to inhibit members of the SMC complexes in cells. We discuss the potential applications of such peptides in research and medicine.

**Session IV: Chromatin Modifiers**

## Forebrain neuronal Smc3 regulates appetite, weight, and metabolic health partially through the regulation of melanocortin 4 receptor

Natalia Saleev[1], Dmitriy Getselter[1], Evan Elliott[1]

1. The Azrieli Faculty of Medicine, Bar-Ilan University, Safed, Israel.

SMC3 is a major component of cohesin complex that regulates higher-order chromatin organization and gene expression. Human genetic studies reveal that de novo mutations in *SMC3* gene, found in patients with Cornelia de Lange syndrome (CdLs). This syndrome is characterized by intellectual disabilities, behavioural patterns as self-injury, as well as metabolic dysregulation. While previous studies have implicated a role for Smc3 in neuronal development, little is known about the exact role of SMC3 in neuronal maintenance and neuronal gene expression in adulthood.
This study aimed to determine the role of SMC3 in adulthood brain, by knocking out Smc3 specifically in adulthood excitatory neurons. Neuron-specific *SMC3* knockout mice displayed a very robust metabolic phenotype in both male and female mice, including an increase in body weight, loss of muscle mass, differences of respiratory exchange, heat production and hormonal changes. The hypothalamus of these mice displayed dysregulated morphology and RNA-seq in the hypothalamus reveals dysregulation in multiple cellular pathways, including a decrease of Melanocortin 4 receptor (Mc4r), a main regulator of appetite. Treatment of these mice with setmenalotide, a MC4r agonist, induced a decrease of weight and food consumption. Therefore, we have identified specific metabolic pathways that are regulated by Smc3 in forebrain neurons, and specific mechanisms that are involved.

**Transcriptional Regulation at DNA Double-Strand Break Sites: A Spotlight on Lysine Crotonylation**

Enas R. Abu-Zhayia, Feras E. Machour, Laila A. Bishara, Bella M. Ben-Oz and Nabieh Ayoub[1]

1. Department of Biology, Technion - Israel Institute of Technology, Haifa 3200003, Israel.

Double-strand breaks (DSBs) at the vicinity of transcriptionally active genes trigger rapid and transient transcriptional silencing. Previously, we showed that CDYL1 is recruited to DNA double-strand breaks (DSBs) to promote homologous recombination (HR) repair and foster transcriptional silencing. Yet, how CDYL1 elicits DSB-induced silencing is not fully understood. Recently, we identified a CDYL1-dependent local decrease in the transcriptionally active marks lysine crotonylation (PanKcr) and crotonylated histone residue H3K9cr at AsiSI-induced DSBs, which correlates with transcriptional silencing. Mechanistically, we revealed that CDYL1 crotonyl-CoA hydratase activity counteracts PanKcr and H3K9cr at AsiSI sites and triggers the eviction of the transcriptional elongation factor ENL to foster transcriptional silencing. Furthermore, genetic inhibition of CDYL1 hydratase activity blocks the reduction in H3K9cr and alleviates DSB-induced silencing, while HR efficiency unexpectedly remains intact. Therefore, our results functionally uncouple the repair and silencing activity of CDYL1 at DSBs. In a broader context, we addressed a long-standing question concerning the crosstalk between HR and DSB-induced transcriptional silencing, suggesting that they are functionally uncoupled and may occur independently.

## A SIR-independent role for Cohesin in subtelomeric silencing and organization

Shikha Laloraya[1]

1. Department of Biochemistry, IISc Bangalore, India

Cohesin is a key determinant of chromosome architecture that is well known for its role in sister chromatid cohesion. It functions as a chromatin organizer due to its DNA binding, tethering, and loop extrusion abilities. Cohesin binds near centromeres, pericentric regions and on chromosome arms at Cohesin Associated Regions (*CARs*) and also close to telomeres, but its role near telomeres remains elusive. In budding yeast, transcription within 20 kb of telomeres is repressed, in part by the histone-modifying Silent Information Regulator (SIR) complex. However, extensive subtelomeric repressed domains lie outside the SIR-binding region, and the mechanism of silencing in these regions remained poorly understood. We have found a role for cohesin in subtelomeric silencing that extends even beyond the zone of SIR binding. Clusters of subtelomeric genes were preferentially derepressed in a cohesin mutant, whereas SIR binding was unaltered. Genetic interactions with known telomere silencing factors indicated that cohesin operates independently of the SIR-mediated pathway for telomeric silencing. Mutant cells exhibited Mpk1-dependent Sir3 hyperphosphorylation that contributes to subtelomeric derepression to a limited extent. Organization of subtelomeric domains and tethering to the nuclear envelope were impaired in cohesin deficient mutant cells. Our findings provide evidence for a unique SIR-independent mechanism of subtelomeric repression mediated by cohesin.

# Thursday, September 7

## Session VI: DNA Damage Response

### Ataxia-Telangiectasia: A Solid Demonstration of the Genome Stability-Aging-Senescence Link

Yosef Shiloh[1]

1. Department of Human Molecular Genetics and Biochemistry, Tel Aviv University School of Medicine, Tel Aviv.

Genome stability is an important determinant of aging pace, and genome instability disorders are often characterized by segmental premature aging. An important aging hallmark is rising amounts of senescent cells. Cellular senescence is a cell state featured by cell cycle arrest for variable periods of time accompanied by marked alterations in cellular shape and metabolism as well as chromatin organization and transcriptome dynamics. Ataxia-telangiectasia (A-T) is a pleiotropic autosomal recessive disorder caused by loss-of-function mutations in the *ATM* gene encoding the homeostatic protein kinase, ATM. A-T is characterized by progressive cerebellar degeneration, immunodeficiency, interstitial lung disease, premature aging, genome instability, cancer predisposition and sensitivity to a variety of DNA damaging agents, most notably those that induce DNA double-strand breaks. A central component of the A-T cellular phenotype is presented by premature senescence of cultured skin fibroblasts from A-T patients. We found that this phenotype is retained under physiological (3%) oxygen concentration despite considerable delay in senescence onset under these conditions. Some transcriptional patterns in senescing A-T fibroblasts were similar to those observed during replicative senescence of control cells, but others were unique to the senescing A-T cells. Murine *Atm*-/- lung fibroblasts exhibited under 3% oxygen premature senescence that was much more rapid compared to that of human A-T fibroblasts. In both ATM-deficient human and mouse cells, a hallmark of genome instability was the elevated amounts of micronuclei, which eventually spill their DNA into the cytoplasm, thereby triggering the cGAS-STING pathway. Indeed, we found that this pathway has a major role in cell senescence induced by ATM deficiency, and characterized its various steps in these cells. Importantly, the results bear implications for some of the cardinal symptoms of A-T. Finally, an update will be provided regarding our long-term follow-up of the health status of A-T carriers. The results indicate a contribution of the *ATM*+/- genotype to aging pace and aging-associated morbidity and demonstrate the effect of heterozygosity for a

null allele at a single locus on public health at large. They also provide further evidence for the important role of genome stability in human aging and the associated diseases.

## Targeting RBM10 deficiency in lung adenocarcinoma

Feras E. Machour[1], Enas Abu-Zhayia[1], Joyce Kamar[2], Alma Sophia Barisaac[1]Itamar Simon[2], Nabieh Ayoub[1]

1. Department of Biology, Technion - Israel Institute of Technology, Haifa, Israel.
2. Department of Microbiology and Molecular Genetics, Institute of Medical Research Israel-Canada, Faculty of Medicine, The Hebrew University, Jerusalem, Israel

Lung adenocarcinoma (LUAD) is a leading cause of cancer-related deaths worldwide[1,2]. The splicing factor RBM10 is commonly mutated in various cancer types and is strikingly one of the most mutated genes in LUAD (~9-25%). Most RBM10 cancer mutations are loss-of-function that correlate with increased tumorigenesis and poor survival and limit the efficacy of targeted therapies in EGFR-mutated lung cancer. Notably, exploiting RBM10 deficiency for targeted cancer therapy has not yet been explored, highlighting the urgency of identifying genetic vulnerabilities to RBM10 loss. Prompted by this, we performed a genome-wide CRISPR-Cas9 synthetic lethal (SL) screen in isogenic LUAD cell line harboring RBM10 cancer mutation and identified 262 high-scoring RBM10 SL genes, including Aurora A and WEE1 kinases. We show that pharmacological inhibition of WEE1 selectively sensitizes RBM10-deficient LUAD cells, including patient-derived cells harboring RBM10 cancer mutations, *in vitro* and in mouse xenograft model. Moreover, the sensitivity of RBM10-deficient cells to WEE1 inhibition is further exacerbated by combined treatment with Aurora kinase A inhibitor. Mechanistically, we demonstrate that the sensitivity to WEE1 inhibition is irrespective of RBM10 splicing activity, and potentiated by DNA damage accumulation, replication stress, and premature mitotic entry. Interestingly, we also reveal an unexpected role of RBM10 in promoting replication fork progression and stress response, at least in part, through its association with replication fork components. Collectively, our data identify DNA replication stress as an SL pathway with RBM10 loss, and provide a repertoire of targets that can be harnessed therapeutically to eradicate RBM10-deficient tumors.

## Alternative repair of DNA double-strand breaks in recombining lymphocytes

Ludovic Deriano[1]

1. Institut Pasteur, Université Paris Cité, Labellisée Ligue Contre Le Cancer, Genome Integrity, Immunity and Cancer Unit, Paris, France

Alternative DNA double-strand break (DSB) end-joining (alt-EJ) is crucial for cell survival in various homologous recombination (HR) and non-homologous end-joining (NHEJ) deficient contexts, where DNA repair is often ensured via the key enzyme Polθ (encoded by Polq in mice). Proper repair of DSBs is essential for adaptive immunity, allowing B lymphocytes to diversify their antigen receptor genes by V(D)J recombination and to switch immunoglobulin heavy chain (IgH) isotypes by the process of class switch recombination (CSR). These recombination events represent the best-known examples of higher vertebrate-specific, NHEJ-dependent physiological processes. During V(D)J recombination, RAG-induced DNA double-strand breaks (DSBs) trigger an ATM kinase-dependent chromatin response and are repaired by components of the NHEJ pathway. During CSR, DNA breaks are induced by the activation-induced cytidine deaminase AID and predominantly repaired by NHEJ in a 53BP1 and Shieldin (SHLD) complex dependent manner. Yet, upon abolition of core NHEJ factors such as XRCC4, LIG4 or Ku B cells maintain the capacity to conduct robust CSR, revealing the existence of alt-EJ pathways which act as safety nets for adaptive immunity. In this talk, I will discuss some of the various parameters that constrain repair of RAG- and AID-DSBs to NHEJ and present recent work on the role of Polθ-mediated alt-EJ in promoting aberrant recombination products during V(D)J recombination and CSR.

**Disrupting translesion synthesis (TLS) activity enhances cell death and prevents tumor growth**

Nomi Barda[1] and Tamar Listovsky[1,2]

1. Department of Molecular Biology, Ariel University, Israel.
2. Adelson School of Medicine, Ariel University, Israel.

DNA-damaging chemotherapy agents such as cisplatin have been a first line approach in cancer treatment for decades; however, their long-term success is often reduced by intrinsic and acquired drug resistance. Although the mechanisms causing drug resistance are quite distinct, they are directly connected to mutagenic translesion synthesis (TLS).
The TLS pathway promotes DNA damage tolerance by supporting both replication opposite to a lesion and inaccurate single strand gap filling. Inhibiting TLS reduces cisplatin resistance and secondary tumor formation. Therefore, targeting TLS is a promising strategy for improving chemotherapy. We recently discovered a small molecule, named c#3, that directly binds to the central TLS protein - MAD2L2, and inhibits TLS activity. Using a mouse model, we demonstrated that combined treatment with cisplatin and c#3 significantly prevented tumor growth. Moreover, c#3 sensitized various cancer cell-lines to cisplatin treatment, co-treatment caused significant elevation in DNA damage levels, explaining the sensitization effect. Using diverse biochemical methods, we demonstrated that c#3 directly binds to MAD2L2 and prevents the formation of the TLS complex in cancer cells. Importantly, the drug is not cytotoxic when administered alone.
Therefore, c#3's activity underlines its potential as a lead compound for developing novel TLS inhibitors for improving chemotherapy treatment reducing the appearance of metastasis and alleviating patients side effect.

**Compaction of chromatin domains by the Smc5/6 complex enhances repair of R-loops**

Damien D'Amours[1], Hemanta Adhikary[1], and Shamayita Roy[1]

1. University of Ottawa, Ottawa, Canada

The structural organization of chromosomes is a crucial feature that defines the functional state of genes and genomes. The extent of structural changes experienced by genomes of eukaryotic cells can be dramatic and spans several orders of magnitude. At the core of these changes lies a unique group of ATPases –the SMC proteins– that act as major effectors of chromosome behavior in cells. The Smc5/6 proteins play essential roles in the maintenance of genome stability, yet their mode of action is not fully understood. We show that the Smc5/6 complex recognizes unusual structures within DNA substrates and uses the energy of ATP hydrolysis to alter the configuration of these substrates in space. This reorganization results in the formation of highly compacted DNA/chromosome subdomains and promotes the repair of R-loops in vivo. Consistent with this, cells defective in the Smc5/6 complex accumulate large quantities of RNA-DNA hybrids in chromatin and show severe synthetic phenotypes when combined with RNA-DNA hybrid detoxification enzymes. Together, our results suggest a novel mechanism for the repair of R-loop lesions in the genome, taking advantage of the DNA compaction activity of the Smc5/6 complex to promote genome stability and organism fitness.

**R-loops and Topoisomerase 1 facilitate formation of transcriptional DSBs at gene bodies of hypertranscribed cancer genes**

Osama Hidmi[1], Sara Oster[1], Jonathan Monin[1] and Rami I. Aqeilan[1]

1. The Concern Foundation Laboratories, The Lautenberg Center for Immunology and Cancer Research, Department of Immunology and Cancer Research-IMRIC, Faculty of Medicine, The Hebrew University of Jerusalem, Jerusalem, Israel

DNA double-stranded breaks (DSBs) pose a significant threat to genomic integrity, and their generation during essential cellular processes like transcription remains poorly understood. In this study, we employed advanced techniques to map DSBs, R-loops, and Topoisomerase 1 cleavage complex (TOP1cc) and re-analyzed ChIP-seq and DRIP-seq data to comprehensively investigate the interplay between transcription, DSBs, Topoisomerase 1 (TOP1), and R-loops. Our findings revealed the presence of DSBs at highly expressed genes enriched with TOP1 and R-loops, indicating their crucial involvement in transcription-associated genomic instability. Depletion of R-loops and TOP1 specifically reduced DSBs at highly expressed genes, uncovering their pivotal roles in transcriptional DSB formation. By elucidating the intricate interplay between TOP1cc trapping, R-loops, and DSBs, our study provides novel insights into the mechanisms underlying transcription-associated genomic instability. Moreover, we establish a link between transcriptional DSBs and early molecular changes driving cancer development. Notably, our study highlights the distinct etiology and molecular characteristics of driver mutations compared to passenger mutations, shedding light on the potential for targeted therapeutic strategies. Overall, these findings deepen our understanding of the regulatory mechanisms governing DSBs in hypertranscribed genes associated with carcinogenesis, opening avenues for future research and therapeutic interventions.

## Unraveling DNA Double-Strand Breaks in Breast Carcinogenesis for Early Detection

Sara Oster[1]

1. The Lautenberg Center for Immunology and Cancer Research, IMRIC, Hebrew University, Israel

DNA double-strand breaks are the most deleterious and can give rise to different genetic aberrations, which later lead to tumor formation and progression. The chain of mutagenic events in breast carcinogenesis following deficient double-strand break (DSB) repair has yet to be characterized via its 'breakome'. Our aim is to characterize early changes in the DNA break pattern of breast cells which can point at potentially pathogenic events early in carcinogenesis. By utilizing utilizing sBLISS (in-suspension break labeling *in-situ* and sequencing), we were able to map sites of recurring DSBs across the genomes of several normal breast and breast cancer samples derived from humans and mice. In addition, we sequenced the 'breakome' of cells derived from healthy women harboring known pathogenic BRCA1/2 mutations. Our data shows a tendency for breaks to accumulate at active regions of the genome, mainly active promoters. Via bioinformatic and web tools, we managed to detect breaks in several gene candidates for further analysis, such as genes related to DNA damage repair mechanisms. Moreover, we observed recurring breaks in *MYC* and *PVT1*, two oncogenes located at a known 'hotspot' for rearrangements in breast cancer, 8q24. Intersection with RNA-seq data revealed a correlation between the 'breakome' and expression, specifically in pathways known to be affected in cancer. Identifying changes in recurring break sites can provide us with insight into the driver events that lead to the initiation and progression of different subtypes or known mutations of breast cancer. Such changes can be later used as clinical 'early detection' biomarkers.

**List of participants**:

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|---|---|
| Kamal Abu Jabal | kamalA@ziv.gov.il |
| Reem Aldwaik | reem.aldwaik@mail.huji.ac.il |
| Wisal Asaad | asaadwisal@gmail.com |
| Ester Avazada | avzada@gmail.com |
| Tal Talya Avitan | Tal.avitan09@gmail.com |
| Orly Avni | orly.avni@biu.ac.il |
| Nabieh Ayoub | ayoubn@technion.ac.il |
| Riham Barazi | Riham.barazi114@gmail.com |
| Marva Bergman | bmarva@gmail.com |
| Laila Bishara | lailab@campus.technion.ac.il |
| Elnatan Bitensky | elnatan.bitensky@mail.huji.ac.il |
| Michael Blank | michael.blank@biu.ac.il |
| Volodymyr Saleev | wolvery232@gmail.com |
| Haim Cohen | Haim.Cohen@biu.ac.il |
| Yuval Cohen | yuval.cohen21@mail.huji.ac.il |
| Aharon Cooper | shmuel5767@gmail.com |
| Shay Covo | shay.covo@mail.huji.ac.il |
| Damien D'Amours | damien.damours@uottawa.ca |
| Ludovic Deriano | ludovic.deriano@pasteur.fr |
| William Earnshaw | Bill.Earnshaw@ed.ac.uk |
| Michal Easton Mor | michal.easton-mor@biu.ac.il |
| Nachshon Egyes | nachshon.egyes@mail.huji.ac.il |
| Rachel Eiges | rachela@szmc.org.il |

| | |
|---|---|
| Hosniyah El Ayoubi | hosniyah.el@mail.huji.ac.il |
| Maria Elias | maria.elias.1007@gmail.com |
| Evan Elliott | Evan.Elliott@biu.ac.il |
| Meital Gal-Tanamy | mgtanamy@gmail.com |
| Osama Hidmi | osamanhidmi@gmail.com |
| Wessal Hanout | wessal224@gmail.com |
| Amnon Harel | amnon.harel@biu.ac.il |
| Itamar Harel | itamarh@mail.huji.ac.il |
| Noa Hourvitz | noa.hourvitz@mail.huji.ac.il |
| Michal Irony-Tur Sinai | michal.irony@mail.huji.ac.il |
| Chizim Iwezor | chizimiwezor@gmail.com |
| Diana Kanaan | diana.kanaan.1998@gmail.com |
| Noam Kaplan | noam.kaplan@technion.ac.il |
| Batsheva Kerem | batshevak@savion.huji.ac.il |
| Shikha Laloraya | slaloraya@iisc.ac.in |
| Dan Lichtental | dan.lichtental@mail.huji.ac.il |
| Erez Lieberman Aiden | erez.lieberman@bcm.edu |
| Feras Machour | ferasmachour@campus.technion.ac.il |
| Kobi Maman | Yaakov.Maman@biu.ac.il |
| Avi Matityahu | Avi.matityahu@biu.ac.il |
| Limor Meoded Danon | limormdanon@gmail.com |
| Antonio Musio | antonio.musio@itb.cnr.it |
| Ron Nagar | nagaron0@gmail.com |
| Sujay Naik | sujaynaik@campus.technion.ac.il |

| | |
|---|---|
| Dania Nassar | danianassar98@gmail.com |
| Monica Neustadter | monica.neustadter@mail.huji.ac.il |
| Itay Onn | Itay.Onn@biu.ac.il |
| Sara Oster | Sara.oster@mail.huji.ac.il |
| Sunil Pachakar | sunilpachakar@gmail.com |
| Avital Parnas | avital.parnas@mail.huji.ac.il |
| Natalia Saleev | natisaleev@gmail.com |
| Meir Shamay | meir.shamay@biu.ac.il |
| Diala Shatleh-Rantisi | dkhs1990@gmail.com |
| Yossi Shilo | yossih@tauex.tau.ac.il |
| Katsu Shirahige | kshirahi@iqb.u-tokyo.ac.jp |
| Yossi Shlomai | josephs@ekmd.huji.ac.il |
| Tania Shubash | taniashubash@gmail.com |
| Riham Smoom | ramsam217@hotmail.com |
| Awakash Soni | soni.awakash@mail.huji.ac.il |
| Layan Tanus | Layan-abr@hotmail.com |
| Moran Titelbaum | moritit le@gmail.com |
| Yehuda Tzfati | tzfati@mail.huji.ac.il |
| Orly Weissberg | ov941908@gmail.com |
| Tamar Yulzary-Listovsky | tamary@ariel.ac.il |
| Aya Zamil | ayazamil1@hotmail.com |
| Hanan Zbedat | zbedathanan@gmail.com |
| Yael Ziv | yaelz@tauex.tau.ac.il |


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