Final aDDRess & CodeAge ITN Conference:

aDDRess & CodeAge Final Annual Meeting

“Molecular Mechanisms in Ageing and Disease"

&

"Funding and Training Opportunities for young researchers"

24- 25 September 2016

Venue: Creta Maris Beach Resort

Hersonissos, Crete

MARIE CURIE ACTIONS

CodeAge
Chronic DNA damage in Ageing

aDDRess

FORTH
INSTITUTE OF MOLECULAR BIOLOGY & BIOTECHNOLOGY

Final aDDRess & CodeAge ITN Conference

Molecular Mechanisms in Ageing and Disease & Funding and Training opportunities for young Researchers

24 - 25 September 2016

Creta Maris Beach Resort
Hersonisos Crete

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1. **Presenter:**
   AGATHANGELOU KYRIACOS

Host Institution: Institute of Molecular Biology and Biotechnology; Foundation for Research and Technology-Hellas, Heraklion, Crete, Greece

Title: The impact of DNA-Damage driven Transcriptional stress in development and disease

DNA damage blocks transcription, affecting gene expression and directly influencing proper genomic administration. Transcription elongation factors help RNA polymerase II (RNAPII) to transcribe past blockages while DNA repair mechanisms constantly preserve the genetic codec's indispensable integrity by repairing lesions (Hoeijmakers, 2001; Wang et al., 2009). Acknowledging the mechanistic synchronization that must be taking place, coordination among DNA repair and arrest-relief is inevitable once RNAPII encounters a lesion. Evidence support the idea of Nucleotide Excision Repair (NER) functioning outside DNA repair, in a subset of genomic administration mechanisms, including transcription, 3d genome organization and chromatin remodeling (Egly and Coin, 2011; Le May et al., 2010). Revealing their involvement in gene expression regulation activities, specific NER factors urge upon an additional level of coordination among the administrators, that necessitates the apportionment of common factors and their functions among these mechanisms. Experimental approaches are directed towards the comprehension of lesion recognition kick-off events for Transcription-Coupled Repair (TC-NER), as well as the delineation of functional complexes that grant synchronization among lesion repair and transcription reboot. Following the generation of BIO-TCEA2 knock in and conditional knock-out mice, TCEA2 associated protein complexes will be initially identified and compared upon UV, with the use of pull-down assays coupled to LC/MS/MS performed on Mouse Embryonic Fibroblasts' (MEFs) and Mouse Primary Dermal Fibroblasts (PMDF) nuclear extracts. In advance, we aim to dissect the functional relevance of DNA damage-driven transcriptional stress in development and disease.

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2. **Presenter:**
   ANKIT ARORA

Host Institution: University of Cologne,Cologne.

Title : RanchNGS: A Web-based Interface for Downstream analysis of ChIP-seq, RNA-seq & Ribo-seq data.

RanchNGS is a web-interface for analysis of sequencing data with respect to biological systems like DNA, RNA and protein. In case of identifying binding sites of DNA associated proteins, our framework uses the sequencing platform of Chip-seq. In case of identifying differentially expressed genes or transcripts it uses the sequencing platform of RNA-seq. In case of monitoring protein translation it uses the sequencing platform of Ribo-seq. We

achieve this goal by using open-source tools and R package repositories from Bioconductor. RanchNGS is easily accessible and user-friendly for biologists for integrative analysis of sequencing data without any prior programming skills.

RanchNGS presents results which are available for download either as plots or tables for further analysis.

3. **Presenter:**
   SEERAT BAJWA

Host Institution: Leibniz Institute on Aging/Fritz-Lipmann Institute (FLI), Jena, Germany

Title: Adaptive immunodeficiency accelerates intestinal aging of telomere-dysfunctional mice

Mice with dysfunctional telomeres suffer from accelerated aging phenotypes. The accumulation of DNA damage triggers checkpoint responses, which compromise stem cell function and prevent genomic instability. However, loss of stem cell function contributes to aging pathologies. Recently, several studies provided evidence that oncogene induced stress and DNA damage both engage the immune system preventing tumour formation and the survival of damaged cells [1]. Little is known about a crosstalk between aging associated DNA damage responses and the immune system. To this end we set up studies comparing aging wild type mice with Rag2 knockout mice, and the respective cohorts in a telomerase (Terc) knockout background. Late generation Terc-/- mice develop age related pathologies in highly proliferative tissues like the hematopoietic system and the intestine characterized by stem cell dysfunction, impaired tissue regeneration, accumulating DNA damage, gastrointestinal crypt atrophy and reduced B and T cell proliferation [2]. Our preliminary data show improved survival of aging late generation Terc-/- mice as compared with double knockout mice (Rag2-/- Terc-/-). The histologic analysis reveals aggravated crypt atrophy, fibrosis, a higher incidence of precancerous microadenoma, and more DNA damage in the gastrointestinal epithelium of Rag2-/- Terc-/- mice compared to Terc-/- mice. FACS analysis revealed increased macrophages infiltration in Rag2 Terc (DKO) lamina propria of intestine. To our knowledge increase macrophages and absence of T cells in Rag2 Terc (DKO) are responsible for wasting colonic phenotype. Innate and adaptive cytokines miscommunication in gut also contributes to pathology. Further experiments are ongoing to confirm these phenotypes mainly by initiation of damage (extrinsic (IR)) and resulting immune cells infiltration in gut lamina propria, detailed sub-phenotyping of infiltrating cells by FACS analysis, at RNA and protein level.

Bajwa S.,Chen Z., Morita Y., Rudolph KL.

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4. **Presenter:**
   COSTIS BOUZALAS

Host Institution: Institute of Molecular Biology and Biotechnology; Foundation for Research and Technology-Hellas, Heraklion, Crete, Greece

Title: The role of XPF in mammalian development

Nucleotide Excision Repair (NER) pathway is a conserved pathway that removes bulky helix-distorting DNA lesions. Whereas defective NER of damaged DNA has been established as the underlying cause of mutations leading to skin cancer, the links between NER defects and the developmental and metabolic abnormalities seen in NER disorders remain obscure. Besides DNA repair, earlier studies have shown that distinct NER factors play also a role in the regulation of gene expression, the transcriptional reprogramming of pluripotent stem cells, the fine-tuning of growth hormones during mammalian development or the biogenesis of ribonucleoprotein complexes. However, the functional contribution of NER to the complex NER developmental disorders remains elusive, primarily due to current difficulties in dissecting the multiple roles of NER proteins in an intact organism. Here, we report the generation of a new series of mice carrying a biotin-tagged version of the NER factor XPF (i.e. bioXPF animals). BioXPF animals were then crossed with mice expressing the BirA transgene; BirA specifically recognizes and biotinylates the short tag, thus creating a very high affinity "handle" for isolating XPF-bound proteins partners by binding to streptavidin. We then identified the proteomic components of XPF-containing protein complexes by recovering proteins from streptavidin-bound nuclear extracts from UV-irradiated Mefs expressing biotin-tagged XPF and analyzing these complexes by mass spectrometry. Next, through biotin-tagged chromatin immunoprecipitations (ChIP) experiments coupled to high-throughput next-generation sequencing, we identified the genomic targets of XPF in transcriptionally induced with trans retinoic acid (tRA) and UV-irradiated MEFs. The identification of novel protein partners of XPF and its genomic targets will aid in the understanding of XPF role in processes beyond NER during mouse development and disease.

5. **Presenter:**
   CHATZINIKOLAOU GIORGINA

Host Institution:Institute of Molecular Biology and Biotechnology; Foundation for Research and Technology-Hellas, Heraklion, Crete, Greece

Title: DNA damage and its impact on developmental gene expression in mammals

Nucleotide Excision Repair (NER) is a major DNA repair pathway assigned with the task to maintain cells genome intact. Inborn NER defects are associated with cancer and premature aging, yet the developmental abnormalities observed in NER patients cannot be explained only by the repair deficiency, suggesting that DNA damage events and genome caretakers may be tightly linked to developmental circuits. To tackle this issue, using an in vivo tagging approach in mice and high-throughput proteomics, we show that the NER structure-specific endonuclease ERCC1-XPF heterodimer interacts with protein factors involved in chromatin organization during mammalian development. Loss of ERCC1 or exposure to various genotoxins triggers the abberant localization of chromatin regulators, altered histone marks and the abberant expression of selected gene targets, requiring functional DNA damage response. Collectively, these data support that persistent DNA damage signaling triggers chromatin changes that affect gene expression programs associated with NER developmental disorders.

6. **Presenter:**
   CHOUDHARY RAMVEER

Host Institution: IFOM - The FIRC Institute Of Molecular Oncology

Title: Chromosome Fragility: A look at Replication Termination.

Replication TERs (termination sites) are major RNA polymerase II and III replication pausing element and cause polar arrest of one of the fork converging at termination zones. Our interest is centered on the processes that coordinate replication with transcription at TERs and on the molecular pathways involved in faithful termination of DNA replication. Leading lagging strand uncoupling followed with Template switching behind the fork would then preserve transcription and copy genetic information from non-transcribing strand. Our aim to understand how the checkpoint machinery, together with HR pathways and specialized DNA helicases (rrm3p and sen1p) and topoisomerases Top2p /condensins prevent chromosome fragility at TERs., thus counteracting unscheduled chromosome rearrangements. A combination of genomic, genetic and molecular biology approaches will be used to analyze the fate of replication forks at TERs in budding yeast. Next generation sequencing will be employ to score for Copy Number Variation (CNVs) to map common fragile site expression. Moreover, EM analysis of replication intermediates will be employed DNA replication in *rrm3* cells is carried on by one or more DNA helicases that

become essential in the absence of Rrm3. *sen1 rrm3* double mutants arrest in the G2/M phase of cell cycle. Using genomic approaches we found that this arrest correlates with the inability to fuse replicons at *TERs* and, consequently *sen1 rrm3* mutants accumulate gaps. We also found that *sen1 rrm3* cells exhibit accumulation of RNA-DNA hybrids at *TERS* and unscheduled condensation events that lead to chromosome entangling. This last phenotype can be rescued by depleting condensins. Furthermore we found that *sen1 rrm3* mutants accumulate anaphase bridges, which might result from attempt to segregate partially, replicated chromosomes. All together our data suggest that, in the absence of Sen1 and Rrm3, cells fail to terminate replication and the unsolved topological constrains cause the premature recruitment of condensins. TERs represent fragile nature mimicking mammalian CFSs, Understanding molecular mechanism at TERs in yeast will help us to dissect governing mechanism in mammals causes genomic rearrangement which is hall mark of cancer.

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7. **Presenter:**
   COSTANZO FEDERICO

Host Institution: IGBMC, Cancer Biology, France

Title: NER factor XPG is recruited genome-wide together with TFIIH to regulate transcription.

The regulation of DNA demethylation is a crucial step for maintenance of cell homeostasis. An active process of DNA demethylation coupled to DNA repair has been related to gene expression. Among the proteins associated to DNA repair, we have demonstrated the implication of the Nucleotide Excision Repair (NER) Factor XPG in transcription. In particular, we already showed that XPG, via its endonuclease activity, can promote DNA breaks and DNA demethylation downstream of retinoic acid (RA) -activated *RARβ2* transcription starting site (TSS) in absence of exogenous genotoxic attack. Nevertheless, our recent results indicate that the accomplishment of active DNA demethylation requires not only XPG but also its known partner TFIIH complex. Hence, this project aims to dissect the roles of XPG coupled to TFIIH in DNA demethylation coupled to transcription by means of genome-wide approaches using cells treated with RA. By ChIP coupled to deep sequencing, we show that XPG is recruited to GC-rich promoters together with TFIIH. Moreover, by comparing cells with or without XPG expression, these XPG/TFIIH targeted promoters displayed differential DNA methylation patterns correlated to deregulation of the expression of the related genes. The determination of the roles played by this NER factors is crucial because mutations in the gene coding this protein originate several genetic disorders also associated with cancer such as Xeroderma pigmentosum (XP), Cockayne syndrome (CS), for some cases combined in XP/CS phenotype. Thereby, the description of the mechanism dependent on XPG in a normal as well as pathological context, is crucial for a better understanding of the aetiology of NER-related and cancer-associated diseases.

8. **Presenter:**
   FABRIZIO D ADDA DI FAGAGNA

Host Institution: IFOM, Italy

Title:DNA damage response activation in cancer and ageing and the role of non coding RNAs

DNA damage and ensuing DNA damage response activation are key players in tumor suppression and ageing. I will discuss how DDR impacts on them and I will discuss a novel and unprecedented role of non-coding RNAs in the modulation of the DNA damage response, including at the telomeres.

9. **Presenter:**
EDIFIZI DILETTA

Host Institution: Cluster of Excellence of the University of Cologne (CECAD), Germany

Title: Proteome Analysis to detect protein profiles changes in NER deficient mutants upon DNA damage in C.elegans

Introduction: Aging is characterized by the declining functioning of tissues and organs and the steadily increased risk of succumbing to aging-associated diseases.The importance of genome maintenance for withstanding the aging process has become particularly evident in a variety of genetic disorders that are caused by heritable mutations in DNA repair genes and are manifested in premature aging in a multitude of tissues [1].

Despite the presence of highly specialized DNA repair systems that maintain genome stability [2], a fraction of DNA damage might persist and lead to increased senescence or cell death with advancing age. Recent work in mice and C. elegans has shed new light on the mechanisms through which developing and aging animals respond to persistent DNA damage.

Results:We are using the nematode C. elegans as a model organism to study how DNA damage accumulation with ageing impinges on pathways involved in longevity assurance. Insulin/IGF-1-mediated signaling (IIS) comprises a conserved longevity assurance pathway and attenuation of IIS activity leads to activation of the FOXO transcription factor DAF-16 resulting in lifespan extension, elevated stress resistance, and enhanced pathogen defense. Previous studies have proposed that DAF-16 functions as a switch, as in the presence of a stress insult, it translocates to the nucleus to delay reproduction and growth while increasing stress resistance and longevity. The activity of DAF-16 is controlled by distinct phosphorylation events that can either activate of inactive DAF-16. In contrast to the DAF-16 activity in the starvation (ST) response, in the presence of UV-induced DNA lesions, the prolonged nuclear translocation of DAF-16 and its activity alleviate the developmental arrest of defective Nucleotide Excision Repair (NER) worms. We used mass-spectrometry-based quantitative proteomics on UV treated L1 larvae which are defective of two key NER genes (xpc-1 and csb-1), to provide a complete picture of the changes occurring at the protein levels upon persistent DNA damage. In parallel we performed a phosphopeptide enrichment using the TiO2 protocol adapted for label free quantitative proteomics, to map the specific post-translational modification (PTM) that can be differentialy regulated upon UV treatment. Proteome and phosphoproteome data were merged into a complex network to unveil new connections and roles played by proteins involved in damage response after DNA damage induction.

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1485.

10. **Presenter:**
GYENIS AKOS

Host Institution: Erasmus MC, Rotterdam , The Netherlands

Title: Deciphering the aging transcriptome by in vivo nascent RNA sequencing

Demographic trends towards an aging society due to continuing increase of life expectancy impose severe pressure on healthcare resources and imply substantial loss of quality of life, urging the need to improve health at older age.

However, development of healthspan-improving interventions requires knowledge about the molecular mechanisms underlying aging. Research using model organisms has highlighted key denominators of aging including a prominent role of persistent DNA damage, originating from endogenous reactive metabolic byproducts. Particularly, mouse models carrying a defect in transcription-coupled DNA repair (TCR), leading to transcriptional stress by damage-induced stalling of RNA polymerases, exhibit reduced lifespan, severe premature but *bona fide* aging phenotypes and gene expression profiles similar to natural aging. It is unknown how transcription-blocking lesions (TBLs) control age-related gene expression, but presumably this results from a combination of transcriptional reprogramming, altered microRNA activity and stochastic accumulation of TBLs affecting gene expression in a gene size-dependent manner. Our main objective is to elucidate how TBLs shape the transcriptome in natural aging as well as accelerated aging in TCR-defective mice. We have developed a highly innovative next generation sequencing method to specifically analyze nascent RNAs labeled in live mice. Using nascent RNA sequencing combined with ribosomal RNA-depleted total RNA sequencing (to measure steady state RNA levels), we will investigate the presence of TBLs in natural aging, analyze how transcription dynamics alters during aging as a result of DNA damage and define transcript classes most vulnerable for acquiring TBLs. These studies will clarify the impact of DNA lesions during age-related gene expression profiles. Simultaneously, this technological breakthrough opens unprecedented perspectives for rapid quantitative analysis of gene expression changes upon differentiation, after exogenous stresses and other environmental or endogenous cues, drug screening and analysis of side effects, etc. and hence will be instrumental for molecular biology in general.

11. Presenter:
HENRIQUES ANA
Host Institution: Biomedcode Hellas SA, Vari, Greece

Title: Role of ERCC1 in Intestinal Inflammation and Tumorigenesis

Cells are constantly exposed to endogenous and exogenous insults that introduce damage into our DNA. To counteract the harmful effects of DNA damage, DNA repair pathways are activated to remove the damage and promote genetic stability. When the DNA repair mechanisms are defective or incomplete, mutations are not excised, leading to genetic instability and risk of hereditary diseases or cancer. It remains yet unknown whether the presence of irreparable DNA lesions may induce chronic inflammation *in vivo*, thereby contributing both to tumour development. ERCC1 (Excision repair cross-complementing group 1) is a molecule that plays an essential role in the NER pathway. Interestingly, ERCC1 polymorphisms have been associated with various types of cancer (e.g. skin and lung cancer), while recently it was also linked to a chronic auto-inflammatory response that causes DNA damage.

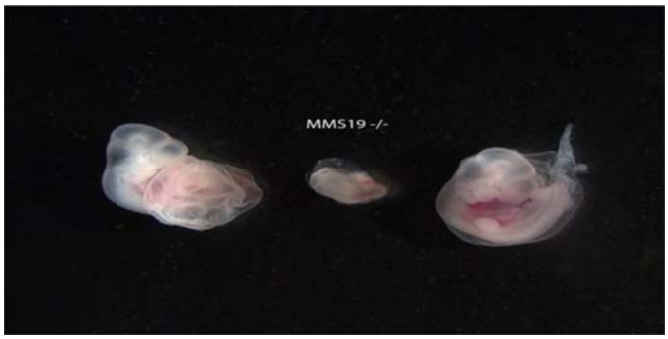
The aim of this project was to determine the role of the NER pathway, and more specifically of ERCC1 in intestinal inflammation, inflammation-driven and spontaneous tumorigenesis. Therefore, we generated mice carrying a deletion of ERCC1 in intestinal epithelial cells (IECs). The mice were viable and fertile, did not exhibit obvious phenotypic defects and showed an efficient and specific deletion of ERCC1 protein in IECs. To study the cell-specific role of ERCC1 in intestinal inflammation, we subjected these mice to the acute and chronic DSS models of colitis. ERCC1 conditional knockout mice show no difference in susceptibility to acute or chronic DSS-induced colitis compared to its control littermates. Furthermore, to assess the role of ERCC1 in inflammation-driven tumorigenesis, we used the well-established AOM/DSS model of colitis-associated cancer. Phenotypic and histological analysis showed no statistical significant differences between the two groups. Finally, we also examined the role of ERCC1 in the $Apc^{min/+}$ model of spontaneous intestinal tumorigenesis and observed that ERCC1 deletion showed no significant difference in the number of macroscopically visible polyps in the intestine compared to their littermate controls.

Collectively, we show with our study that the NER pathway and in particular, deletion of ERCC1 in IECs appears to not play a role in intestinal inflammation, inflammation-driven and spontaneous tumorigenesis.

12. Presenter:
IAMARTINO LUCA
Host Institution: Institute of Molecular Biology and Biotechnology, FORTH, Heraklion, Greece

Title: Transgenic mouse model for NER associated factor: MMS19

MMS19 is a protein involved in Nucleotide Excision Repair (NER), transcription and chromosomal segregation. Most recently, it was shown that MMS19 assembles iron-sulfur proteins required for DNA metabolism and genomic integrity [1, 2]. Yeast mms19 (MET18) deletion mutant cells are sensitive to DNA-damaging agents revealing so a NER defect; moreover the deletion renders the cells temperature-sensitive for growth [3]. At present, despite the iron-sulfur metabolism, any mechanistic understanding of how MMS19 functions in transcription and DNA repair remains elusive. To extend the analysis in a systemic level, my colleagues and I are generating a transgenic mouse model.



We have attempted to generate a full knock-out Mms19 animal, but, unexpectedly, the total depletion of MMS19 resulted to be embryonically lethal as shown in the following figure.

Using the Cre-loxP recombinase strategy, we have designed a Mms19 transgenic construct with floxed locus. In particular, the first exon is flanked by two loxP sites that will drive its excision upon the expression of the Cre recombinase. The floxP Mms19 mice will be crossed with transgenic mice with tissue specific Cre expression. The offspring generated will be Mms19 -/- tissue specific that will be used for extensive and multidisciplinary studies to dissect the contribution of Mms19 in tissue homeostasis and organ development.

Currently, we have been working on targeting of mouse embryonic stem cells that, after selection and genotyping, will be implanted in mice foster mothers. Those will give birth to chimeras that will be crossed each other to generate full knock-in transgenic mice (floxP Mms19).

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13. Presenter:

KIDIYOOR GURURAJ RAO

Host Institution: IFOM- FIRC Institute of Molecular Oncology, Milan, Italy

Title: Role of ATR in regulating nuclear plasticity and cell migration

ATR is key Kinase involved in maintaining genome integrity, in sensing DNA damage and initiating the DNA damage response. Complete depletion of ATR causes embryonic lethality in mice models and hypomorphic mutations in humans are associated with the autosomal recessive disorder called Seckel syndrome.

We have previously reported that ATR mediates a response to mechanical stress by relocating at the nuclear envelope and Nucleoli(*Kumar et al. Cell 2014*). We now show that depletion of ATR alters cellular and nuclear morphology and alters the overall cell stiffness. The nucleuses of ATR depleted cells, accumulated both type I and type II nuclear envelope invaginations. All these results of ATR depleted cells in nuclear and cell plasticity suggest that ATR has a role in regulating plasticity of cell. Further, ATR defective cells were assayed in variety of migratory tests including wound healing, invasion in collagen matrix and migration through constrictions. Results from these assays confirm that cells lacking ATR exhibit plasticity problems and are defective in migration.

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14. **Presenter:**
    KOUTSIOULIS DIMITRIS

Host Institution: Institute of Molecular Biology and Biotechnology; Foundation for Research and Technology-Hellas, Heraklion, Crete, Greece

Title: Tips on starting a business in science

How difficult is it to transform the opportunity and the novelty of your idea into a successful company? Creating a bio-related product is vastly different from other industry products; however, there are some key topics that remain universal. A series of steps that you need to follow before preparing your own business plan and approaching potential investors will be discussed.

As an example of how to start a business in science, MINOTECH biotechnology developmental stages will be presented. MINOTECH biotechnology (Mb) is the in-house facility of IMBB-FORTH for the production of high value biotechnology products. Mb products are directed and supplied to scientists engaged in Research, Biotechnology Industry and Clinical Laboratory. Taking advantage of IMBB new policy to encourage entrepreneurship Mb assets were further exploited. Mb is under ISO 9001.2008 certification and has vast know-how and methodologies for sustaining large scale production of bacterially derived products. Mb redesigned mission and vision is to target molecular biology market with a more aggressive-innovative behavior.

15. **Presenter:**
    LIGNER JOACHIM

Host Institution: Swiss Institute for Experimental Cancer Research (ISREC), School of Life Sciences, Ecole Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland

Title: Telomere troubles during chromatin damage and oxidative stress

Telomere damage and shortening has been implicated in aging, cancer development and in telomeropathies in which patients suffer from bone marrow, lung or liver abnormalities. Continuous telomere shortening occurs in cells that lack telomerase due to the end replication problem. In addition, stochastic telomere loss events can occur due to difficulties of the semiconservative DNA replication machinery in replicating telomeric DNA. Furthermore, telomeric DNA appears particularly sensitive to chemical damage possibly due to the G-tracts in telomeric repeats or because telomeres suppress certain DNA repair pathways. I will report on how telomeres may compensate for their enhanced vulnerability to oxidative stress by recruiting the oxygen radical scavenger peroxiredoxin 1 (PRDX1) to telomeric chromatin. We find that PRDX1 depletion leads to oxidative damage of telomeric DNA. Furthermore, we identify specific defects of telomerase when attempting to elongate oxidized DNA substrates. Our results indicate that PRDX1 preserves telomeres from damage and in good condition for elongation by telomerase. Telomere shortening leads to reduction of TRF2 at chromosome ends triggering the telomeric DNA damage response (DDR) and cellular senescence. At has been proposed that the telomeric DDR is linked to t-loop unfolding[1] or to decompaction of telomeric chromatin[2]. I will report on our analysis of telomeric chromatin size and shape by STORM microscopy in wild type cells and cells that had been depleted of TRF2. Our data demonstrate that the telomeric DDR is not linked to chromatin decompaction.

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[1]Doksani et al. Cell (2013) 155: 345-56

[2]Bandaria et al. Cell (2016) 164: 735-46

16. **Presenter:**
    LYDALL DAVID

Host Institution n: Institute for Cell and Molecular Biosciences University of Newcastle

Title: Telomere duplication and protection

Telomeric DNA, at the ends of eukarotyic chromosomes, is difficult to replicate and vulnerable to recognition by the DNA damage response machinery. Consequently telomeric DNA sequences are comparatively unstable. The repetitive, non-coding, nature of telomeric DNA helps cells tolerate changes in telomeric DNA structure. I will discuss some of our latest work aimed at understanding how telomeric DNA is replicated and stabilized in budding yeast.

17. **Presenter:**
    MAJERSKA JANA

Host Institution: School of Life Sciences, Swiss Institute for Experimental Cancer Research, Lausanne, CH-1015, Switzerland

Title: Telomeres in cancer development

Telomeres - special chromatin structures that cap and protect the ends of human chromosomes - have been widely implicated in the cellular processes of ageing and cancer. Several lines of evidence suggest that cancer development might be accompanied by reorganization of telomeric chromatin. However, a systematic, comprehensive study of the tumorigenesis-associated changes in telomere protein composition is still missing. We have applied the Quantitative telomeric chromatin isolation protocol (QTIP) [1, 2] to compare telomeric states in isogenic cell lines representing several stages of the transformation process. Using the approach developed by Hahn. et al. [3, 4], human embryonic lung fibroblasts were converted into tumorigenic cells in a step-by-step fashion using serial introduction of genes encoding the hTERT subunit of telomerase, the SV40 large T and small T antigens, and the H-RasV12 oncogene. Pairwise comparison of the four cell lines has revealed transformation-induced alterations in abundance and/or telomere occupancy of multiple proteins, including some unexpected protein networks. Currently, we are investigating the biological relevance of these findings. Besides, several novel telomeric proteins have been identified and are being validated.All in all, this project may open up novel avenues for investigating the roles of telomeres in cancer, which in turn could facilitate the development of telomere-based prognostic, diagnostic and/or therapeutic strategies for oncology patients.

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18. Presenter:
MARKIEWICZ-POTOCZNY MARTA
Host Institution: Institute for Cell and Molecular Biosciences, Newcastle University, Newcastle upon Tyne

Title: Is the essential function of Dna2, a conserved nuclease/helicase, in telomere biology?

Aims/Objectives: The aim of the project was to identify and understand the DNA damage response (DDR) networks using model organism *Saccharomyces cerevisiae*. *DNA2,* which encodes a conserved and essential helicase/nuclease, is involved in DNA replication, Okazaki fragment maturation and DNA resection during double strand break (DSB) repair. In mammalian cells DNA2 preferentially associates with telomeres during replication and has a role in G-quadruplex DNA cleavage. Deletion of *DNA2* is lethal, but three mechanisms are known to suppress the lethality of *dna2*Δ in yeast: overexpression of $RAD27^{FEN1}$, a nuclease involved in Okazaki fragment processing, deletion of *PIF1*, a helicase involved in long flap formation during DNA replication, or deletion of *RAD9*, a DNA damage checkpoint protein [1].*Methods:* Standard molecular and genetic approaches (e.g. PCR, cloning, Southern blot, gene deletion, yeast mating, transformation, sporulation and tetrad dissection, serial dilution and growth on plates) were applied to create and test yeast strains containing multiple mutations in genes involved in checkpoint response. Synthetic Genetic Array (SGA) was used to combine a genome wide collection of gene deletions with *dna2*Δ mutation. *Results:* We report that, in contrast to published data, deletions of other checkpoint genes (*ddc1*Δ*, rad17*Δ*, chk1*Δ*)* suppress the lethality of *dna2*Δ mutants at the level similar to that of *rad9*Δ mutation. Double mutants initially grow poorly but get fitter with time. Such a pattern of growth (initial sickness and recovery over time) is also observed in strains with defects at telomeres. Indeed, a Southern blot analysis of *dna2*Δ cells confirms telomeres are longer in such cells. SGA results confirm that *ddc1*Δ*, rad17*Δ*, chk1*Δ suppress *dna2*Δ and comprise a list of other genes that regulate *DNA2* function.**Conclusions:** It has been proposed that the essential function of Dna2 is in Okazaki fragment processing and lagging strand synthesis. However, we observe that checkpoint gene deletions suppress *dna2*Δ, whereas they exacerbate defects in other core DNA replication proteins [2]. Therefore we propose that the essential function of Dna2 is in telomere maintenance, rather than in general chromosome replication. Our model fits with published data showing that Dna2 binds and is important at telomeres in mammalian cells, and is specifically required for maturation of telomeric DNA replication intermediates in yeast. Our results suggest that Dna2, like the CST complex, might be a telomere-focused DNA replication protein.

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19. Presenter:
MUESSAR J. KRISTOFER
Cyagen Biosciences and Vectorbuilder.com

Title: A Director's guide to navigating the Life Sciences Industry

While genetically engineered mouse/rat models have been used extensively in biomedical research, the design and generation of these models is often complicated, time-consuming and costly. In this presentation, one will provide a brief overview of our transgenic and knockout mouse and rat services (www.cyagen.com). This includes traditional pronuclear injection based transgenics, our new PiggyBac transgenic mouse service, ES cell homologous recombination based gene targeting, and CRISPR-mediated genome editing technology. One will introduce our newest ES-cell based gene targeting technology - TurboKnockout®, which allows the generation of conditional knockout/knockin mouse models within 6-8 months, at least 4-6 months shorter than the standard approach. One will also introduce our revolutionary new custom vector construction web-based tool www.Vectorbuilder.com,

which was designed to make the tedious process of cloning obsolete. Finally, one will discuss possible career choices for graduate students and post-docs to pursue in the life sciences industry. Using personal experiences one will show what possible professional pathways are available to those who receive an advanced degree in the life sciences.

20. Presenter:
NIKOLETOPOULOU VASILIKI
Host Institution: Institute of Molecular Biology and Biotechnology; Foundation for Research and Technology-Hellas, Heraklion, Crete, Greece

Title: The role of autophagy in synaptic plasticity

Autophagy is a catabolic pathway that is crucial for neuronal integrity, yet, its regulation in the brain remains elusive. Similarly, while it is widely accepted that protein degradation is required for synaptic plasticity, the contribution of autophagy is still unknown. Our findings reveal that BDNF signaling suppresses the autophagic machinery *in vivo*. In addition, suppression of autophagy is required for BDNF-induced synaptic plasticity. Finally, we identify 3 postsynaptic scaffold proteins as synaptic cargo of autophagy. These findings place autophagy alongside the ubiquitin-proteasome system and protein synthesis, as a novel component of protein turnover at the synapse that is required for remodeling of synapses.

21. Presenter:
NTARI LYDIA
Host Institution: Biomedcode Hellas S.A., Vari, Greece

Title: NER and arthritis-related pathologies

Introduction**:** Rheumatoid arthritis is a chronic inflammatory condition characterized by inflammation of the joints leading to cartilage destruction and bone erosion, often accompanied by comorbidities such as heart pathologies. The mesenchymal-origin synovial fibroblasts (SFs) have a crucial role in the initiation and development of arthritis pathology [1]. SFs from hTNF-overexpressing transgenic mice [hTNFTg (Tg197)] that develop spontaneous arthritis are characterized by a specific activated/arthritogenic phenotype [1,2]. Mutations in ERCC1 protein (Nucleotide-Excision-Repair family) cause severe progeroid syndrome in humans and mice, as well as inflammatory responses in specific cell types, such as adipocytes [3]. As adipocytes are of mesenchymal origin, we hypothesized that Ercc1 defect may also interfere with other mesenchymal cell-dependent pathologies such as arthritis and its comorbidities.
Results: To evaluate the effect of defective NER in the development of arthritis, we used either progeroid Ercc1 mutant (Ercc1$^{-/\Delta}$) or mesenchymal cell specific Ercc1KO (ColVI*Cre*/Ercc1f/-) mice. Clinical and histopathological evaluation of arthritis pathology did not reveal any signs of inflammation, bone erosion or cartilage destruction in either mutants. *In vitro* evaluation of SFs phenotype did not show similarities to the arthritogenic Tg197 SFs (increased adhesion, migration and proliferation) coming to an agreement with the *in vivo* results. In the absence of a spontaneous arthritic phenotype, arthritis was either induced or genetically imposed to ColVI*Cre*/Ercc1f/- animals. Interestingly, mesenchymal deletion of Ercc1 resulted in partial amelioration of the Collagen-Antibody-Induced-Arthritis symptoms, while crossing of ColVI*Cre*/Ercc1f/- mice with the Tg197 spontaneous arthritis model, left arthritis pathology unaffected. The arthritis pathology was similarly unaffected in Tg197/ Ercc1$^{-/\Delta}$ mice. However, left sided heart valve pathology, an arthritis comorbidity that develops with 100% penetrance in Tg197 mice, was still present upon mesenchymal deletion of Ercc1 but was completely abolished in Tg197/Ercc1$^{-/\Delta}$ mice. Characterization of this pathology revealed its dependency on the mesenchymal-origin Valve Interstitial Cells (VICs) that share a common "activated" phenotype to arthritogenic SFs (hTNF secretion, high migratory and proliferative ability). Amelioration of this pathology in the Tg197 Ercc1$^{-/\Delta}$ mice, suggests a modification of the activated VICs phenotype that is currently under investigation.
Perspectives: Our results show that Ercc1 defect does not lead to the development of spontaneous arthritis pathology. On the other hand, induction of arthritis in MSC specific deletion of Ercc1 leads to amelioration of induced arthritis pathology while systemic Ercc1 deletion leads to amelioration of heart pathology in Tg197 mice. Interpretation of these results will lead to a better understanding of the complex interactions between NER and mesenchymal cell-dependent pathologies.

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22. Presenter:
PAPANDREOU ELENA MARGARITA

Host Institution: Institute of Molecular Biology and Biotechnology; Foundation for Research and Technology-Hellas, Heraklion, Crete, Greece

Title: DNA damage-induced autophagy and necrotic neurodegeneration during ageing

Accumulation of DNA damage is a key determinant of ageing and has been implicated in neurodegeneration. Although it is well known that ultraviolet (UV) radiation induces apoptosis, the contribution of necrotic cell death to DNA damage-related pathology remains elusive. To address this question, we developed a nematode model for DNA damage-induced neurodegeneration by using UV-C irradiation to trigger DNA damage in *C. elegans* neurons. Initial observations using this model show a marked increase of cytoplasmic calcium concentration upon UV irradiation. To examine whether this acute cytoplasmic calcium elevation triggers necrosis in neurons, we exposed DNA repair-defective mutants to UV light. These mutant animals are hypersensitive to UV irradiation and exhibit widespread necrotic cell death in somatic tissues upon exposure, while neurons are particularly affected. Runaway autophagy has previously been implicated in necrotic neurodegeneration. In this context, we investigated the contribution of autophagy in DNA damage-induced cellular pathology and nuclear dynamics. Notably, we found that DNA damage induces autophagic flux and alters nuclear dynamics both in nematodes and mouse cells. We are currently dissecting the interplay between DNA damage-induced autophagy, nuclear membrane alterations and necrotic cell death, aiming to identify evolutionarily conserved molecular mechanisms interfacing these processes.

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23. Presenter:
PRINCZ ANDREA

Host Institution: Institute of Molecular Biology and Biotechnology; Foundation for Research and Technology-Hellas, Heraklion, Crete, Greece

Title: SUMOylation regulates lifespan and mitochondrial homeostasis through DAF-16/FOXO in *C. Elegans*

The nematode *Caenorhabditis elegans* is an ideal model organism for studying the biology of ageing; the pathways modulating the ageing process are well-characterized and conserved. Amongst these signaling pathways, insulin/IGF-1 has a major role in determining the lifespan of animals, mainly through the DAF-16/FOXO transcription factor and the stress response-related transcription factor SKN-1/NRF2. Interestingly, these two key transcription factors contain putative SUMOylation sites. SUMOylation, the attachment of SUMO (small ubiquitin-related modifier) to a protein, is a posttranslational modification implicated in the regulation of diverse cellular processes, including the DNA damage response, sub-cellular protein localization and protein-protein interactions, among others. Protein SUMOylation levels increase progressively during ageing. However, whether elevated SUMOylation is only an unrelated consequence of the ageing process or it serves a causative, regulatory role in senescent decline is not understood. *Results:* The *C. elegans* genome contains a single gene encoding SUMO (*smo-1*), rendering the nematode a convenient model in which to genetically dissect the role of SUMOylation in organismal physiology and ageing. Deletion of *smo-1* causes embryonic lethality. Nevertheless, we find that RNAi knockdown of *smo-1* initiated at the L4 stage shortens the lifespan of both wild type and long-lived animals.

Neuron-specific knockdown of *smo-1* does not alter median lifespan. Notably, knockdown of the SUMO protease gene (*ulp-1*), extents the lifespan of long-lived mutants (*clk-1*, *daf-2*, *ife-2*), but not wild type animals. In addition, we observed that manipulation of SUMOylation levels by either knockdown of *smo-1* or *ulp-1* influences the activity of DAF-16 and SKN-1, as well as, stress resistance, mitochondrial homeostasis and energy metabolism, in a genetic background- and age-dependent manner. *Conclusion:* Perturbing SUMOylation alters the lifespan of wild type and long-lived mutant animals. SUMOylation also regulates the intestinal mitochondrial network through DAF-16. We are currently investigating the possible tissue-specific and cell non-autonomous mechanisms by which SUMOylation modulates the activity of key, stress response transcription regulators, and how these mechanisms interface with main signalling pathways that impinge on longevity.

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24. Presenter:
RODRIGUES JOANA

Host Institution: Institute for Cell and Molecular Biosciences, Newcastle University Medical School, Newcastle upon Tyne, United Kingdom

Title: PAF1 complex components regulate TERRA and telomere function

A functional telomere depends on interactions between hundreds of different proteins and telomeric nucleic acids. Telomerase, DNA-binding and DNA damage response proteins are just some of many classes of protein that affect telomere function. The conserved PAF1 complex (Cdc73, Paf1, Ctr9, Leo1 and Rtf1, in yeast), which affects RNA abundance in eukaryotes, affects telomere function in yeast. Here we have systematically investigated the roles of PAF1 complex components on telomere biology. We inactivated individual components of the PAF1 complex and measured cell fitness, telomere structure, transcriptional silencing, TERRA (telomeric repeat containing RNA) and other RNA levels. From these experiments we conclude that individual PAF1 complex components perform different functions at telomeres. We show that loss of Cdc73 improves fitness of telomere defective yeast cells, while loss of other PAF1 components have the opposite effect. We also show that Paf1 and Ctr9 strongly reduce TERRA, while Cdc73, Leo1 and Rtf1 have little effect. Paf1 and Ctr9 function independently of Sir4 to regulate TERRA levels and this is because they stimulate TERRA decay, as well as decay of other RNAs. We propose that effects of individual PAF1 components on TERRA and telomerase RNA levels are critical for affecting cell fitness. We suggest that high levels of TERRA, in combination with low levels of telomerase, observed in *paf1*Δ and *ctr9*Δ cells are particularly detrimental to cell fitness. In contrast, *cdc73*Δ mutants have equally low levels of telomerase but normal TERRA levels, and are comparatively fit. The different effects of individual PAF1 components on telomere biology in human cells may help explain why Cdc73, in particular, is affected in human cancer.

25. Presenter:
SABATELLA MARIANGELA

Host Institution: Department of Genetics, Erasmus MC, Rotterdam

Title: *In vivo* function and regulation of ERCC1-XPF.

The structure-specific endonuclease ERCC1-XPF is essential to incise DNA lesions in Nucleotide Excision Repair (NER) and Interstrand Crosslink (ICL) repair. In humans, defects in ERCC1-XPF can affect several DNA repair pathways therefore leading to different phenotypes: cancer predisposition, severe developmental defects and accelerated aging. Although the function of ERCC1-XPF has been studied in great detail in biochemical and cell biological experiments, it is not entirely clear how the activity of this complex in response to different kind of lesions is regulated and how its deficiency can lead to such a variety of tissue-specific symptoms.

To determine how ERCC1-XPF is specifically regulated in response to UV and ICLs, we stably expressed fluorescently tagged ERCC1-XPF in different mammalian cell lines and identify its interactome by quantitative proteomics. This confirmed the known association with NER core factors XPA, XPG and TFIIH specifically after UV damage. We also identified several putative novel interactors, which are currently being investigated. Furthermore, we set up new imaging methods to monitor the recruitment of ERCC1-XPF to both UV-lesions and intra- and interstrand crosslinks and to evaluate the involvement of (novel) regulatory proteins. Results so far confirm that indeed XPA and FANCD2 promote the recruitment of ERCC1-XPF to DNA damage. Previously, our lab showed that ERCC1/XPF loss-of-function in *C. elegans* causes development defects and accelerated aging, reminiscent of human patients. To study potential tissue-specific activities of ERCC1-XPF, we expressed and imaged fluorescently tagged ERCC1-XPF in the *C. elegans* germline, hypodermis, intestine, neurons and muscles. Preliminary results show that in oocytes the complex quickly but only transiently re-localizes to damaged chromosomes upon UV irradiation. Moreover, we find that the mobility of the complex differs depending on chromatin content and cell type. By combining genetic analysis, imaging and proteomic screening, we aim to uncover the regulatory mechanisms that underlie damage and cell type specific responses of ERCC1-XPF.

Mariangela Sabatella, Karen Thijssen, Wim Vermeulen and Hannes Lans
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26. Presenter:
SAREDI GIULIA

Host Institution: Biotech Research and Innovation Centre (BRIC) and Centre for Epigenetics, Faculty of Health and Medical Sciences, University of Copenhagen

Title: H4K20me0 marks post-replicative chromatin and recruits the TONSL-MMS22L DNA repair complex

After DNA replication, chromosomal processes including DNA repair and transcription take place in the context of sister chromatids. While cell cycle regulation can guide these processes globally, mechanisms to distinguish pre- and post-replicative states locally remain unknown. Here we reveal that new histones incorporated during DNA replication provide a signature of post-replicative chromatin, read by the human TONSL–MMS22L homologous recombination complex. We identify the TONSL ankyrin repeat domain (ARD) as a reader of histone H4 tails unmethylated at K20 (H4K20me0), which are specific to new histones incorporated during DNA replication and mark post- replicative chromatin until the G2/M phase of the cell cycle. Accordingly, TONSL–MMS22L binds new histones H3–H4 both before and after incorporation into nucleosomes, remaining on replicated chromatin until late G2/M. H4K20me0 recognition is required for TONSL–MMS22L binding to chromatin and accumulation at challenged replication forks and DNA lesions. Consequently, TONSL ARD mutants are toxic, compromising genome stability, cell viability and resistance to replication stress. Together, these data reveal a histone-reader-based mechanism for recognizing the post-replicative state, offering a new angle to understand DNA repair with the potential for targeted cancer therapy.

Giulia Saredi[1], Hongda Huang[2]*, Colin M. Hammond[1], Constance Alabert[1], Simon Bekker-Jensen[3], Ignasi Forne[4], Nazaret Reverón-Gómez[1], Benjamin M. Foster[5], Lucie Mlejnkova[6], Till Bartke[5], Petr Cejka[6], Niels Mailand[3], Axel Imhof[4], Dinshaw J. Patel[2] & Anja Groth[1]*

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27. **Presenter:**
SCHIAVONI FEDERICA

Host institution: CNIO, Programma de Oncologia Molecular, Spain

Title: New insights into the roles of the SMC-5/6 complex in DNA repair

The SMC-5/6 complex is part of the Structural Maintenance of Chromosomes (SMC) family of proteins, which includes condensin and cohesin and whose main function is the regulation of chromosomal architecture and organization. Several roles have been proposed for SMC-5/6 in genome maintenance, but its specific function is still unclear. Using a collection of mouse models of a SUMO ligase named NSMCE2, which is an integral part of the SMC5/6 complex, we have now seen that NSMCE2 is essential for mammalian cells, and that the complex suppresses cancer and ageing in mice. In addition to overall the accelerated ageing that appears when NSMCE2 is deleted in adult mice, these animals have a distinct phenotype on their kidneys. Given the recent connections between DNA damage, replication stress and kidney disease, part of my PhD is now to explore the roles of NSMCE2, and the SMC5/6 complex, in the kidneys. An overview of our overall work on mouse models of the SMC5/6 complex, plus some early data on the connection between NSMCE2 and kidney disease will be discussed.

Authors: Federica Schiavoni, Emilio Lecona, Ariana Jacome and Oscar Fernández-Capetillo

28. **Presenter:**
SILVA CRISTINA RIBEIRO

Host institution: Department of Genetics, Erasmus MC, Rotterdam

Title: SWI/SNF ATPases BRM and BRG1 stabilize TFIIH via its GTF2H1 subunit and are essential for Nucleotide Excision repair

Nucleotide Excision repair (NER) removes a wide range of structural DNA lesions that distort the DNA double helix, such as the damage inflicted by UV light. For optimal genome maintenance, NER must be capable of accessing lesions everywhere in the genome at any moment, regardless of chromatin conformation. However, it is not precisely known how chromatin is modified to ensure optimal removal of NER specific lesions. Also, the role that chromatin modifiers - such as chromatin remodelers - have in the facilitation of the DNA damage response is not yet clear. We have previously identified proteins of the SWI/SNF chromatin remodeling family complex as essential factors for optimal UV-survival in *C. elegans*. To characterize the function of SWI/SNF complexes in NER, we focused on its two ATPases subunits, BRM and BRG1, and aimed to determine whether – and how – these proteins facilitate the repair of UV-DNA damage by NER. We now show that the mammalian SWI/SNF ATPases have a critical role in regulating NER efficiency early after damage is induced, both in Global Genome and Transcription-Coupled NER. We found that BRM and BRG1 facilitate loading of the general transcription factor TFIIH complex, which is required for unwinding the DNA helix and verify damage for NER. Consequently, when BRM and BRG1 are depleted, loading of downstream repair factors on DNA damage is also impaired. Subsequent analysis, such as of protein stability and expression, led us to conclude that both SWI/SNF ATPases, BRM and BRG1, determine TFIIH complex stability via transcriptional regulation of the core subunit GTF2H1/p62.

Our data suggests that the involvement of chromatin remodelers in DNA repair goes beyond providing access of detection proteins to DNA damage. Due to transcriptional regulation of GTF2H1/p62, SWI/SNF ATPases are crucial for maintaining stability of TFIIH proteins, which is not only essential for NER, but is also indispensable for basal transcription to take place. Moreover, as SWI/SNF proteins are often found to be mutated in different types of cancer, our results suggest that such mutations may have serious consequences to DNA damage response in cancer cells.

Cristina Ribeiro-Silva, Özge Z. Aydin, Wim Vermeulen, Hannes Lans

29. Presenter:
SHROFF MAITHILI

Host Institution: MRC Protein Phosphorylation Unit, University of Dundee

Title: Characterization of a putative DNA repair nuclease, C15ORF41

Congenital dyserythropoietic anaemia (CDA) consists of a heterogeneous group of very rare hereditary disorders characterized by distinct morphological abnormalities of the erythroid precursors in bone marrow, ineffective erythropoiesis and suboptimal reticulocyte response [1, 2]. CDA has been defined by 3 major subtypes, as classified by Heimpel & Wendt [3]: CDA-I, CDA-II and CDA-III. Congenital dyserythropoietic anaemia type I (CDA-I) is characterized by moderate to severe macrocytic anaemia, hepatomegaly, spongy heterochromatin and inter-nuclear bridges. A vast majority (~80%) of the known cases of CDA type I disease have been found to be associated with mutations in the CDAN-1 gene [4-6]. Mutations (substitutions) identified using a complete genome study [7] in the previously uncharacterized locus, C15ORF41 suggests a possible causative gene underlying the CDA type I disease. This could address the ~20% of CDA-I cases lacking any CDAN1 mutations. Structural analysis of the protein provided evidence of 2 N-terminal AraC/XylS-like helix-turn-helix domains followed by a PD-(D/E)XK nuclease domain, implying C15ORF41 is a nuclease. The PD-(D/E)XK superfamily of proteins exhibits sequence divergence and variable structural elements interspersed within a relatively small and evolutionary conserved core which makes it difficult to identify new members of this family. However, several diverse and previously uncharacterized proteins (including C15ORF41) have been identified using bioinformatics studies [8].

Based on these findings, we hypothesize that C15ORF41 may play a role in chromatin reassembly during replication. We present the ongoing studies to test C15ORF41 function and to understand the basis of pathogenesis associated with mutations in C15ORF41 and congenital dyserythropoietic anaemia type I.

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30. Presenter:
SPANOU CHARA

Host institution: Cluster of Excellence of the University of Cologne (CECAD)

Title: Coelomocytes in regulating programmed cell death on *C.elegans* germline

Cells employ various strategies to tackle genomic instability. Some of these processes are regulated by cell cycle checkpoints, which arrest the cell cycle to allow time for damage repair or, when the damage is irreparable, promote senescence or apoptosis. In addition, apoptosis on *C.elegans* germline seems to be triggered as a defense mechanism

against *Salmonella typhimurium* infection. The coelomocytes are three pairs of terminally differentiated cells that are in fixed positions within the pseudocoelomic space. They are predicted to serve immune or detoxification functions. Via their robust endocytic activity, they engulf substances from the body cavity fluid. To clarify the potential role of coelomocytes in regulating DNA damage-induced programmed cell death, coelomocyte-deficient worms were exposed to ionizing radiation (IR) and germ line apoptosis was quantified. To clarify the potential role of coelomocytes in worm immunity, coelomocyte-deficient worms were infected with a strain of pathogenic *E.Coli* and apoptosis on the germline was assessed after infection. Programmed cell death is crucial for clearing damaged cells after DNA damage and is also vital for worm survival after *Salmonella typhimurium* infection. We are currently testing the hypothesis that DNA damage-induced programmed cell death might not be entirely cell autonomous and also that programmed cell death might be triggered on the coelomocyte-deficient *C.elegans* germline as a redundant mechanism to protect the worm against infection.

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Cluster of Excellence of the University of Cologne (CECAD), Cologne, Germany

31. Presenter:
TSOUROULA KATERINA
Host Institution: IGBMC, Cancer Biology, France

Title: Double Strand Break repair mechanisms in heterochromatin revealed with the CRISPR/Cas9 system

DNA double strand breaks (DSBs) are among the most harmful DNA breaks because their unfaithful repair can lead to chromosomal translocations and consequently cancer. DSB repair functions in the context of highly-ordered chromatin structure. Heterochromatin (HC), the stably compacted part of chromatin, is highly condensed, restricting DNA transactions and thus rendering DSB repair a challenging process that cells need to overcome to preserve genome integrity. Using the CRISPR/Cas9 system to specifically and robustly generate DSBs in pericentric heterochromatin of mouse fibroblasts, we have shown that in G1, breaks are repaired by NHEJ at the core of these heterochromatic domains. On the contrary, in G2, DSBs occur at the core but they are repaired at the periphery of the HC domain by HR. Mechanistically, this DSB-relocalization at the periphery requires end resection and exclusion of RAD51 from the core. Our goal is to gain insight into the mechanism that excludes RAD51 from heterochromatin. To investigate this question, we will create two differentially tagged RAD51 knock-in cell lines allowing us to isolate proteins or protein modifications that block RAD51's entrance in HC by co-immunoprecipitation experiments and subsequent analysis and identification of the candidates by mass spectrometry. This project will reveal the molecular mechanism of RAD51's exclusion and thus HR restriction outside of HC in mammals.