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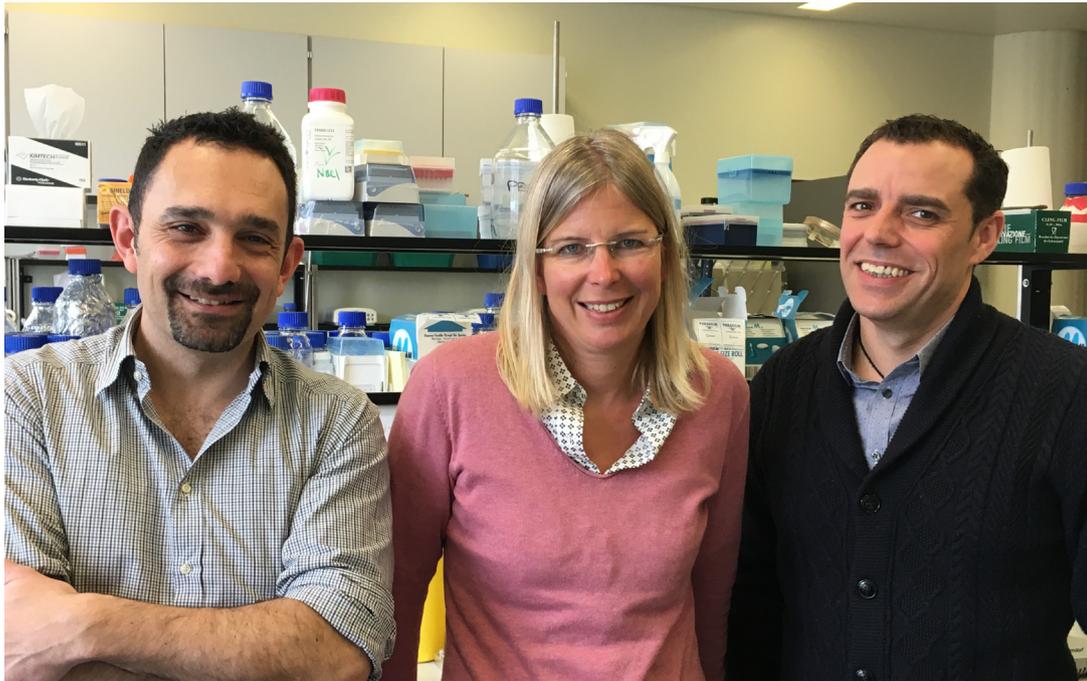
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THE BOARD OF DIRECTORS



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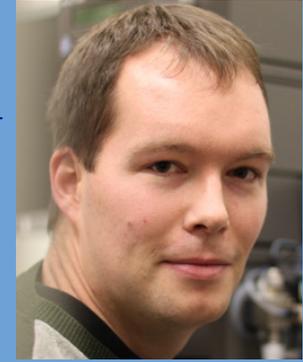
FOREWORD

The IMCR in 2017 has been home to eight research groups comprising approximately 70 students, post-docs, and technical and administrative staff. The Institute was restructured in February of 2017, when its previous director, Prof. Dr. Josef Jiricny retired and moved to ETH. Our new leadership consists of three tenured faculty, Profs. Anne Müller, Massimo Lopes and Alessandro A. Sartori, who together form the Board of Directors and were appointed by the faculty of Medicine to this function in 2016. The Board is presided by the Chair of the Board, currently Prof. Müller, who serves in this position for two years on a rotating principle. The IMCR is one of several basic research institutes and clinics (among them: the Institutes of Experimental Immunology, Anatomy, Physiology and Biochemistry, and Clinics of Dermatology, Hematology, Oncology, and Radio-oncology of the Medical Faculty, along with the Institutes of Molecular Biology of MNF and Department of Disease Mechanisms of Vetsuisse) that in 2017 have launched the Cancer Research Center (CRC) of the University of Zurich. The mission of this large consortium, which currently comprises around 50 PIs, is to (1) foster academic and scientific excellence in cancer research in Zurich, to (2) bridge basic/preclinical science and clinical research, to (3) enable rapid translation of novel discoveries into clinical trials and to (4) establish Zurich as a major international center in this field. The University and Medical faculty governing bodies have made Cancer Research a research priority and together are providing the funds required to kick-start the CRC. The IMCR sees itself at the center of CRC activities, and we are proud that our PIs serve as founding members of this consortium. Together with our colleagues from the Department of Disease Mechanisms of Vetsuisse, we form one

of four research pillars that have been identified as strongholds of cancer research in Zurich, i.e. Genetics, Epigenetics and Genomics (alongside stem cell biology, tumor immunology and oncogenic signaling). While we continue to envision ourselves as an Institute with a shared mission of understanding molecular mechanisms of carcinogenesis, we strive to increasingly integrate our efforts with those of our CRC colleagues at the University hospitals to generate synergies and work on problems that are relevant from a clinical perspective.

The main focus of research at the IMCR remains the study of genomic instability in cancer. The individual research programs revolve primarily around the enzymes involved in the metabolism of DNA, ranging from DNA repair proteins, through helicases and exonucleases to proteins involved in the repair of double-strand breaks and in DNA damage signaling. The latter area was further strengthened by the arrival of our latest group leader, assistant professor Lorenza Penengo, who studies post-translational modifications affecting members of the DNA damage response pathways. DNA repair is of particular relevance when the response of human cells to a wide range of substances used in the therapy of cancer is analyzed. The group of Anne Müller brings another dimension to our research portfolio, and, although its research program has links to the other projects currently running in the institute, the main focus of the group is on chronic inflammation in cancer, especially on the role of *Helicobacter pylori* infection in gastric malignancies, as well as on lymphoma pathogenesis research.

PETR CEJKA



RECOMBINATION MECHANISMS



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*left 2016

Our laboratory is interested in the molecular mechanisms of homologous recombination. Recombination is the principal pathway for the repair of broken DNA, but also for generation of allele combinations that promote genetic diversity. We are using mostly biochemical techniques to study the action of recombination proteins on intermediates of DNA metabolism. As radiation and many drugs used to treat cancer cause DNA damage and specifically DNA breaks, our work is also relevant for understanding the mechanisms of chemo- and radiotherapy.

Petr Cejka and his group moved to the Institute for Research in Biomedicine (Bellinzona) associated with the Università della Svizzera italiana at the end of September, 2016.

Deoxyribonucleic acid (DNA) stores genetic information that contains instructions for the proper development and function of all living organisms. The integrity of DNA must be preserved during the life cycle in order to maintain cellular functions and to pass information encoded in it onto the next generation. It has been estimated that each cell in a human body acquires tens of thousands of DNA lesions per day. Throughout evolution, cells have evolved a number of DNA repair pathways specialized for the various classes of DNA damage. Our interest in these mechanisms is stimulated by the fundamental importance these processes play in life. Many DNA repair factors are essential for viability – cells cannot exist without them. Others are important only in special cases - hereditary or sporadic defects in some components of the repair machinery lead to a variety of syndromes characterized by premature aging, cancer predisposition or other abnormalities. Finally, the efficiency of DNA repair mechanisms often affects cancer chemotherapy: a number of drugs that are being used to treat cancer act by causing DNA damage. The mechanisms of action of these drugs as well as repair strategies are often not well understood.

Our research group is interested in DNA repair mechanisms from a basic research standpoint: we want to learn how these pathways operate in healthy cells and how defects lead to abnormalities and disease. Specifically, we focus on a DNA repair pathway termed homologous recombination. Homologous recombination is a highly intricate complex of processes, which repairs breaks in DNA strands. Most cells contain more than one copy of genetic information in each cell, and homologous recombination can exploit that in a very elegant manner. It can restore the integrity of the damaged DNA molecule by using genetic information stored in the identical (or homologous) copy of DNA. This process may thus restore DNA integrity in a largely accurate manner. Homologous recombination is highly conserved in evolution: the mechanism in the bacterium *Escherichia coli* or in the yeast *Saccharomyces cerevisiae* is very similar to the mechanism in human cells. This observation underlines the fundamental importance of this pathway in all kingdoms of life. Also, by using the simple organisms as research models, we can learn about homologous recombination in an experimentally more feasible setup. Our research group is using both *Saccharomyces cerevisiae* and human systems.

Currently, we are using mostly biochemical methods to answer fundamental questions in biology. We express and purify recombinant proteins, assemble multiprotein complexes, and analyze their be-

havior on synthetic structures that mimic their physiological substrates. Genetic and cell biological techniques are suitable to identify components of new or existing pathways and phenotypic analysis often infers a specific function, yet it becomes limiting when trying to elucidate detailed molecular mechanisms. Redundant and overlapping pathways often further complicate interpretation of in-vivo based experiments. Biochemical analysis is in contrast very powerful to explain underlying molecular mechanisms.

Recombination in DNA replication: Promoting genome stability

Lucie Mlejnkova, Lepakshi Ranjha

In addition to repair double-stranded DNA breaks, homologous recombination helps to stabilize or restart replication forks in the presence of single-stranded DNA breaks or replication-blocking lesions. This likely represents the most important function of recombination, as recombination-deficient human cells can undergo only a very limited number of rounds of DNA replication. The link between stalled or collapsed replication forks and recombination is not understood. It has been inferred that the human MMS22L-TONSL complex might function in this process, but the underlying mechanism is unclear. We could show that MMS22L-TONSL binds RPA-coated single-stranded DNA, which may help recruit the complex to sites of DNA damage. By a direct interaction with the strand exchange protein RAD51, MMS22L-TONSL promotes DNA strand exchange by limiting the assembly of RAD51 on double-stranded DNA. The activity of MMS22L-TONSL then promotes replication fork reversal to protect stalled or stressed replication forks. We further investigated how MMS22L-TONSL functions together with RAD51 to promote replication fork stability, as well as how this function interplays with other factors including SMARCAL1 and ZRANB3.

Replication fork repair by recombination must be tightly regulated so that it is only activated when needed. Unscheduled DNA recombination might lead to sister chromatid exchanges, loss of heterozygosity, genome rearrangements and other abnormalities, and must be thus tightly controlled. The ultimate goal of our experiments is to understand how MMS22L-TONSL regulates recombination specifically upon replication fork stalling. Our research is anticipated to shed light on the link between DNA replication and repair.

Promotion of genetic diversity in meiosis: resolution of recombination intermediates

Roopesh Anand, Lepakshi Ranjha, Nicolas Weyland

Promotion of genetic diversity is a key function of sexual reproduction. At the molecular level, this is controlled by the homologous recombination machinery, which exchanges (recombines) DNA fragments between the maternal and paternal genomes. During this process, joint molecules form between the 'mum' and 'dad' chromosomes, leading to intermediates termed double Holliday junctions. These joint molecules are then processed in a way that results in the physical exchange of genetic information between the two recombining chromosomes. This so-called crossover is an integral and essential part of the meiotic cell division. Results from genetic, cell biological and cytological experiments identified the Mlh1-Mlh3 heterodimer as part of a protein complex that is required for the generation of crossovers during meiotic homologous recombination. However, the mechanism of this reaction is completely unknown. The aim of our research is to analyze the behavior of the purified recombinant Mlh1Mlh3 complex as well that of its partners in the processing of double Holliday junctions. We want to show how Mlh1-Mlh3 can cleave these structures into exclusively crossover recombination products, and therefore explain the molecular mechanism underlying the generation of diversity in meiosis.

So far, we successfully expressed and purified the yeast Mlh1-Mlh3 and human MLH1-MLH3 recombinant proteins into near homogeneity. We could show that the recombinant MutL γ is indeed a nuclease that nicks doublestranded DNA in the presence of manganese, similarly to the mismatch repair specific MutL α nuclease. MutL γ binds DNA with a high affinity, and shows a marked preference for Holliday junctions, in agreement with its anticipated activity in their processing. Specific DNA recognition has never been observed with any other eukaryotic MutL homologue. Mismatch repair specific MutL α shows no binding preference to mismatched DNA. MutL γ thus represents a new paradigm for the function of the eukaryotic MutL protein family. We further collaborate with the Valerie Borde group (Curie Institute, Paris) to define the function of meiotic recombination proteins.

Funding

Swiss National Science Foundation, Swiss Cancer League

First steps in homologous recombination: DNA end resection

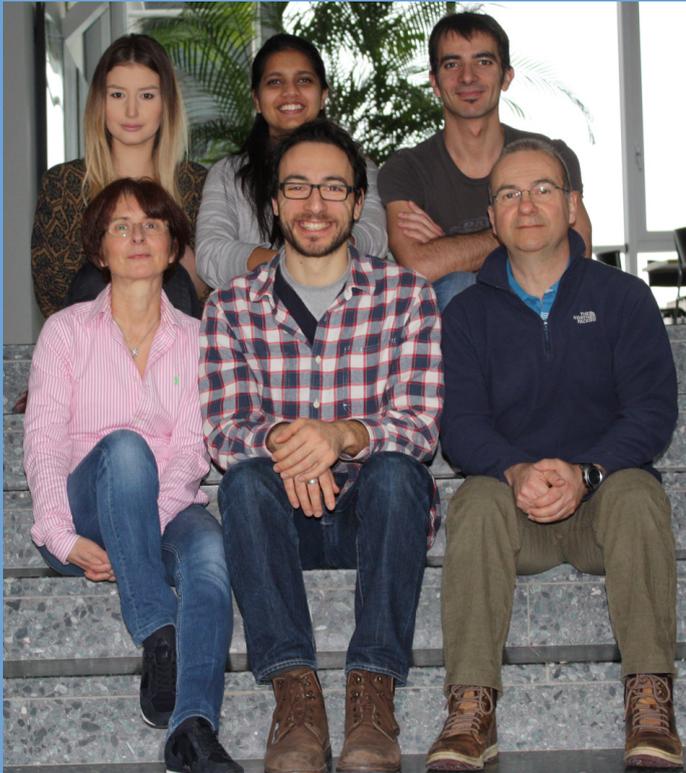
Roopesh Anand, Elda Cannavo, Sean Michael Howard, Maryna Levikova, Cosimo Pinto

Homologous recombination is initiated by the nucleolytic degradation (resection) of the 5'-terminated DNA strand at the DNA break. This leads to the formation of 3'-tailed DNA, which becomes a substrate for the strand exchange protein RAD51 and primes DNA synthesis during the downstream events in the recombination pathway. DNA end resection thus represents a key process that commits the repair of DNA breaks into recombination. Research from multiple laboratories established that DNA end resection is in most cases a two-step process. It is initiated by the nucleolytic degradation of DNA that is at first limited to the vicinity of the broken DNA end. This is carried out by the Mre11-Rad50-Xrs2 (MRX) complex and Sae2 proteins in yeast, and MRE11-RAD50-NBS1 (MRN) and CtIP proteins in human cells. We could reconstitute these reactions *in vitro*, and demonstrated that Sae2 and CtIP stimulate a cryptic endonuclease activity within the yeast MRX or human MRN complex, respectively. The activity of Sae2/CtIP is absolutely dependent on its phosphorylation. The reconstituted DNA clipping reaction allows us to investigate the mechanism of this process as well as its regulation by posttranslational modifications and additional protein co-factors. Specifically, we investigate how phosphorylation stimulates Sae2 and CtIP on the mechanistic level. We also study how the endonuclease and exonuclease activities of the MRE11-RAD50-XRS2/NBS1 complex are regulated by the individual subunits and other proteins, including the BRCA1-BARD1 complex.

Downstream of MRX-Sae2 and MRN-CtIP, which process only a limited length of DNA, DNA end resection is further catalyzed by Sgs1-Dna2 or Exo1 in yeast and BLM-DNA2, WRN-DNA2 or EXO1 in human cells. We specifically investigated the Dna2/DNA2 enzyme. We could show that both yeast Dna2 and human DNA2 possess a cryptic helicase activity, which is masked by its nuclease domain. We observed that the helicase activity of Dna2/DNA2 contributes to the degradation of unwound DNA by its partner helicase (Sgs1, BLM, WRN), which overall promotes the efficiency of DNA end resection, and therefore homologous recombination.

STEFANO FERRARI

DNA DAMAGE AND THE CELL CYCLE



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* left 2016

Faithful execution of the cell division cycle ensures correct transmission of genetic information to the progeny, preventing the development of cancer. Damage occurring to DNA during transition through the cell cycle is generally addressed before mitosis. To this end, cells are equipped with dedicated protein complexes that orchestrate DNA repair while triggering checkpoints that arrest the cell cycle. Activation and suppression of checkpoints rely on rapid switches consisting of reversible post-translational modification (PTM) of proteins, including phosphorylation, ubiquitylation and sumoylation.

Control of yeast Exonuclease-1 by phosphorylation

Giuseppe De Gregorio

On one front, my laboratory devotes significant efforts to elucidate basic mechanisms that govern the cellular response to DNA damage. Clarifying how activation of DNA repair pathways influences the onset of mitosis will lead to the identification of new checkpoint elements and to the definition of novel gene interactions, offering targets for therapy.

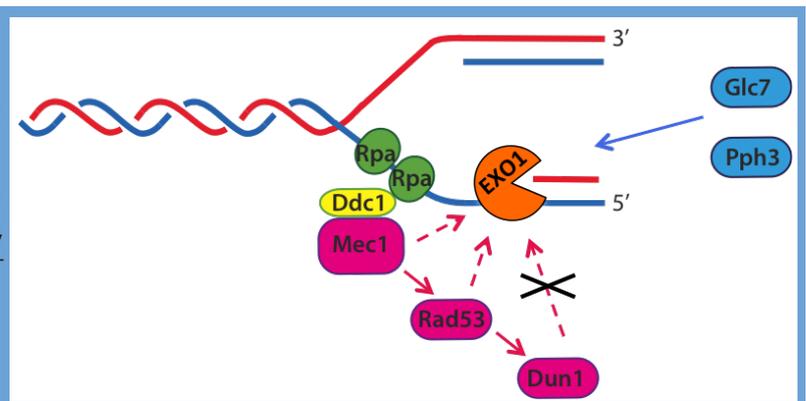
On another front, my laboratory is engaged in the discovery of small molecule compounds targeting established components of signalling pathways that are hyperactivated in cancer. Rationale to this approach is the consideration that increased survival of cancer patients treated with aggressive radio- or chemotherapy comes at a price, with secondary leukemia being one of the most common causes of death. The rational design of molecules targeting oncogenic pathways that are hyper-functional in cancer cells or to which cancer cells become addicted has provided an alternative and promising approach to the use of “dirty” drugs. However, the established adaptability of cancer cells to external insults constantly demands implementation of novel strategies.

EXO1 is a Rad2 family DNA repair nuclease able to remove mononucleotides from the 5' end of the DNA duplex. EXO1 was originally identified in *S. pombe* and subsequently in humans and was shown to participate in several post-replication DNA repair pathways including mismatch repair, DSBs repair, meiotic and mitotic recombination. Additionally, yeast Exo1 was shown to localize at stalled replication forks where it counteracts fork reversal.

We have previously shown that, in response to stalled DNA replication, human EXO1 is regulated by phosphorylation-dependent poly-ubiquitylation, which results in its proteasome-mediated degradation to limit long range DNA resection, whereas yeast Exo1 is regulated through interaction with 14-3-3 proteins, possibly mediated by phosphorylation.

In this study, we set out to identify the entire complement of phosphorylation sites in yeast Exo1 as well as kinase and phosphatase controlling Exo1 phosphorylation upon stalled DNA replication. So far, we have identified 14 sites of phosphorylation in yeast Exo1, five of which are targeted *in vitro* by Rad53. Moreover, Exo1 phosphorylation in response to HU results to be Mec1/Rad53-dependent but Dun1-independent and the protein phosphatases Glc7 and Pph3 are the most likely candidates for Exo1 dephosphorylation during checkpoint recovery (Fig. 1). *In vivo* DNA resection assays that we are conducting upon expression of Exo1-5A-mutant in *exo1Δ sgs1Δ* strains will allow directly and unequivocally assessing the effect of phosphorylation on Exo1 activity.

Figure 1. Phosphorylation-dependent control of yeast Exo1 at stalled replication forks. Upon stalled DNA replication, Exo1 is phosphorylated in a Mec1/Rad53-dependent but Dun1-independent manner at five major sites. During checkpoint recovery, Exo1 dephosphorylation is likely controlled by the protein phosphatases Glc7/Pph3.



Mitotic ATPases and genome stability

Christian Gentili, Ishita Tripathi

RUVBL1 and RUVBL2 are essential genes belonging to the AAA⁺ superfamily (ATPases Associated with various cellular Activities). The two polypeptides form homo- and hetero-hexamers and are part of large multiprotein complexes involved in chromatin remodeling and transcription. RUVBL1 and RUVBL2 expression is upregulated in cancer.

In a study focused on RUVBL1, we have shown that prolonged suppression of RUVBL1 expression by RNAi causes an abnormal extension of prometaphase and severe defects in chromosome segregation, with polar chromosomes failing to align at the metaphase plate and ultimately lagging at anaphase, a condition that leads to aneuploidy. Considering the multiple roles of RUVBL1/RUVBL2 in the cell cycle, in a follow-up study we conceived a system to ablate the RUVBL1 protein in G₂, allowing us to more precisely elucidate the mitotic function of the RUVBL1/RUVBL2 complex. To

this end, we used cells stably expressing a plant E3 ubiquitin ligase that, upon selective activation by the plant hormone Auxin, ubiquitylates proteins carrying a defined “degron” sequence, causing their degradation by the proteasome (Fig. 2). We inserted the specific degron sequence at either the 5' or 3' of RUVBL1 using CRISPR/Cas9 technology and obtained evidence that both constructs underwent rapid degradation upon addition of Auxin. With this tool in hand and using time-lapse microscopy, we now plan to deplete RUVBL1 in G₂ and examine length of metaphase, abnormalities in chromosome congression and presence of lagging chromosomes. Mechanistically, we can assess whether RUVBL1 is involved in polar ejection of chromosomes at metaphase (i.e., through interaction or modulation of function of motor proteins such as CENP-E) and determine the ability of RUVBL1 to affect kinesin-dependent microtubule motility. Furthermore, we intend to investigate the role RUVBL1 in determining proper localization of SAC components at kinetochores in relation to the lagging chromosome phenotype observed in our published studies.

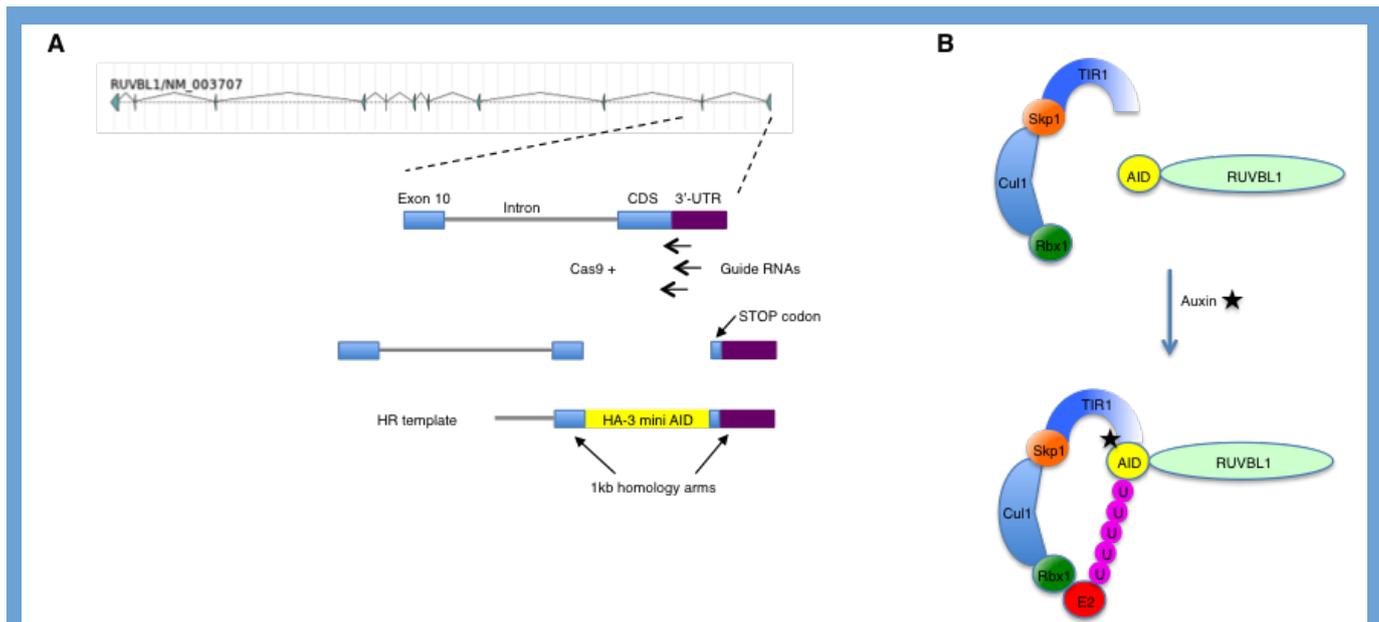


Figure 2. Schematic representation of the Auxin-inducible degron system.

A. Three minimal auxin-inducible degron sequences (AID) are positioned in frame to RUVBL1 using CRISPR-Cas9 technology (in this case the C-terminal construct is shown with the position of 3 possible guide RNAs). B. Addition of plant hormone auxin (black asterisk) causes rapid TIR1-dependent ubiquitylation and degradation of the fusion protein carrying the degron.

Discovery of novel small molecule inhibitors of the dual specificity phosphatase CDC25

Zeynep Kabakci, Christiane König, Christian Gentili

CDC25 phosphatases have an established role in cell cycle transitions and have been recognized as important targets for cancer therapy. Previous drug discovery programs identified distinct classes of CDC25 inhibitors. However, none of the compounds so far described has attained approval, failing to keep up with expectations. In this study, we set out to discover novel CDC25 inhibitors using a combination of computational approaches as well as biochemical and cellular assays. Upon defining a minimal common pharmacophore in established CDC25 inhibitors of three distinct molecular groups, we performed a virtual screening of a proprietary library. Next, taking advantage of the availability of crystal structures for

CDC25A and CDC25B and using a molecular docking strategy, we carried out hit expansion/optimization. Enzymatic assays revealed that naphthoquinone scaffolds were the most promising CDC25 inhibitors among selected hits. At the molecular level, the identified compounds acted through a mixed-type mechanism of inhibition of phosphatase activity, involving reversible oxidation of the catalytic cysteine. In 2D cell cultures, the compounds caused arrest of the cell cycle at the G1/S or at the G2/M transition. Analysis of mitotic markers and time-lapse microscopy revealed that the compounds effectively impaired CDK1 activation and function and that mitotic arrest was followed by cell death (Fig. 3). Finally, studies on 3D organoids derived from intestinal crypt stem cells of *Apc/K-Ras* mice revealed that administration of the compounds at low-doses caused arrest of proliferation and induction of differentiation markers.

The lead compounds reported in our study open interesting perspectives to the design of novel anti-cancer therapeutics targeting CDC25.

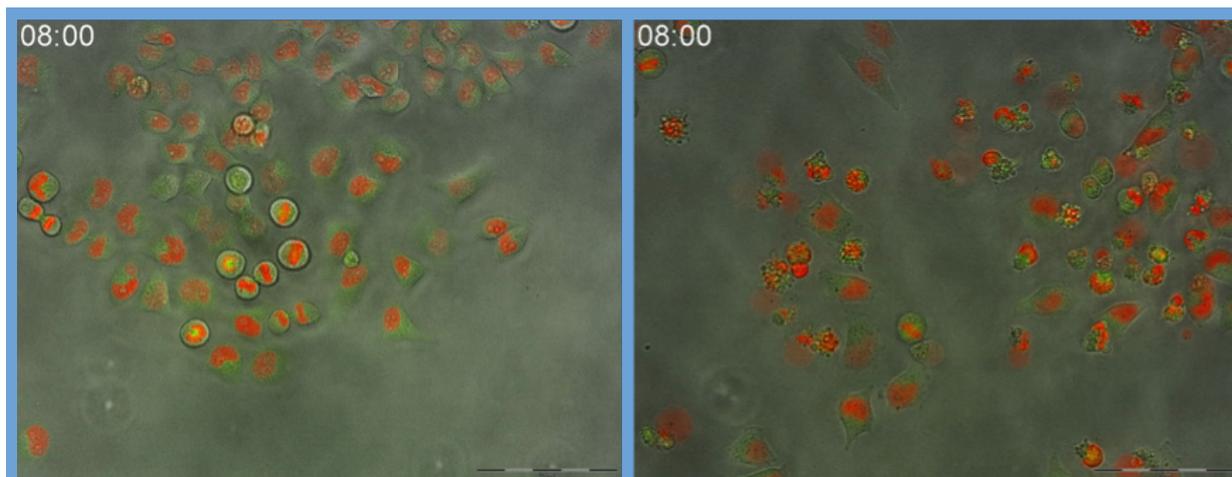


Figure 3. Effect of the CDC25 inhibitor UPD-790 on cell transiting through mitosis. Fluorescence microscopy still images of synchronized Kyoto HeLa cells (mCherry-H2B / GFP-tubulin) transiting through mitosis. Cells were treated with vehicle alone (left) or UPD-790 (5 μ M).

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KERSTIN GARI



IRON-SULPHUR PROTEINS AND GENOME STABILITY



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In recent years, several proteins essential for DNA replication and repair have been identified to require binding to an iron-sulphur (FeS) cluster for their function. Given that upon FeS cluster oxidation, free iron can potentially generate DNA-damaging reactive oxygen species, these discoveries have come as a surprise and the function of FeS clusters in these processes is still poorly understood. We are using a combination of techniques in order to understand how genome stability is maintained, with a special emphasis on the role of FeS clusters in the processes of DNA replication and repair.

Iron-sulphur clusters in DNA replication and repair proteins

Stanislaw Jozwiakowski, Richard Lutz, Diana Odermatt, Sandra Kummer, Sebastian Wild

Iron-sulphur (FeS) clusters are ancient and versatile cofactors that are found in a variety of proteins throughout all kingdoms of life. They are most commonly known for their role in electron transport in the mitochondrial respiratory chain, but they are also found in the active sites of a variety of enzymes. The most striking feature of FeS clusters is their ability to adopt redox potentials over a wide range, which is based on the fact that iron can switch between two oxidation states (+2 and +3). A common motif is the so-called [4Fe-4S] cluster, in which four iron atoms and four sulphur atoms sit in the corners of a cube-like structure, which is incorporated into an FeS protein through coordinative binding by four cysteine residues. For a long time, it appeared that nuclear FeS proteins with affinity for DNA are relatively rare. However, recent evidence suggests

that a considerable number of DNA replication and repair factors require binding to an FeS cluster for their function, amongst them the DNA helicases RTEL1, XPD, FANCI and ChIR1, and proteins essential for DNA replication, such as DNA primase and DNA2. With the discovery that all three replicative polymerases in yeast are FeS proteins, it has become clear that FeS clusters in DNA replication and repair proteins are the rule rather than the exception (Figure 1). However, the actual function of FeS clusters in these proteins is still very poorly understood. Owing to their redox sensitivity, FeS clusters are particularly interesting and versatile cofactors that would be uniquely suited as regulatory cofactors that could e.g. sense oxidative stress conditions during DNA replication. One attractive possibility is that FeS clusters could serve as intrinsic stress sensors within DNA replication and repair proteins and directly modulate their biochemical activities to allow adaptation to suboptimal conditions of DNA replication.

We are using a combination of techniques, such as cell biology, molecular biology and biochemistry, in order to address the function of FeS clusters in DNA replication and repair proteins.

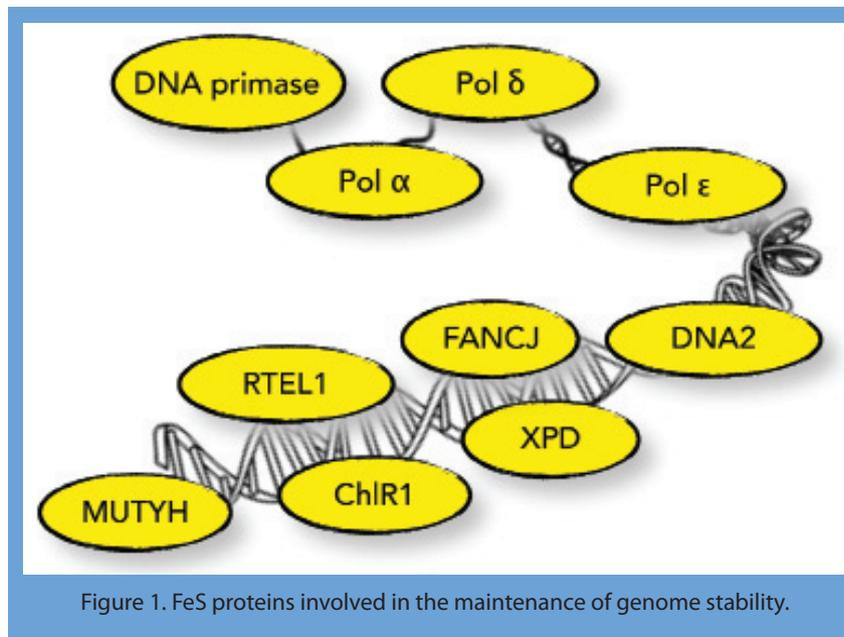


Figure 1. FeS proteins involved in the maintenance of genome stability.

Biogenesis of nuclear FeS proteins

Diana Odermatt

The maturation of nuclear FeS proteins starts in mitochondria and is finished in the cytoplasm with the aid of the multi-protein cytoplasmic iron-sulphur assembly (CIA) machinery. The actual transfer of an FeS cluster to FeS client proteins is carried out by the CIA targeting complex composed of MMS19, MIP18 and CIAO1.

We could show that MIP18 and CIAO1 associate with the C-terminus of MMS19. By doing so they form a docking site for FeS proteins that is disrupted in the absence of either MMS19 or MIP18. The FeS helicase XPD seems to be the only exception since it can interact with MMS19 independently of MIP18 and CIAO1. We further show that the direct interaction between MMS19 and MIP18 is required to protect MIP18 from proteasomal degradation. Taken together, these data suggest a remarkably regulated interaction between the CIA targeting complex and client proteins, and raise the possibility that FeS cluster transfer is controlled, at least in part, by the stability of the CIA targeting complex itself.

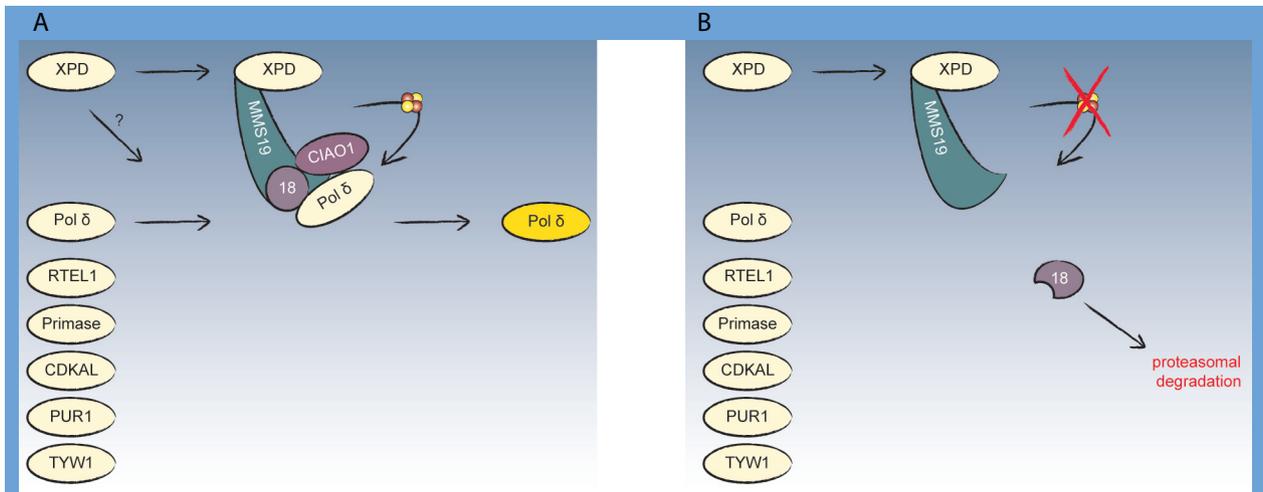


Figure 2. The CIA targeting complex.

(A) MIP18 and CIAO1 interact with the C-terminal HEAT repeats of MMS19 and form a docking site for FeS proteins to bind to and FeS cluster transfer to occur. In contrast to other FeS proteins, XPD can interact with the N-terminal HEAT-repeats of MMS19 independently of MIP18 and CIAO1. It may or may not associate with the C-terminus of MMS19 in addition. (B) When binding of MIP18 to MMS19 is impaired or in a situation when MMS19 levels are low, MIP18 is not protected by MMS19 anymore and gets targeted for proteasomal degradation (from Odermatt and Gari, *Cell Reports*, 2017).

Causes and consequences of DNA replication stress

Bartłomiej Porebski, Anna Simon, Sandra Kummer, Sebastian Wild

Maintaining the integrity of the genome is of outmost importance for a cell to remain functional, and in turn genome instability is one of the hallmarks of cancer cells. DNA replication stress is an important source of genome instability, and signs of it are already found at the earliest stages of cancer development.

In recent years, it has become clear that encounters between the DNA replication and transcription machineries are a major challenge during DNA replication and a cause of genome instability. During these encounters, incompletely processed RNA molecules can form persistent RNA:DNA hybrids (so called *R-loops*) that need to be removed for replication to proceed. To date the mechanism of how R-loop removal is coordinated with replication fork progression has remained poorly understood. Using a combination of biochemistry and cell biology we investigate how R-loops are unwound during DNA replication.

Although the causes of DNA replication stress are diverse, a general consequence of DNA replication stress is the remodelling of stalled replication forks, presumably to provide time and space for clearance of the replication block. Many aspects of the transactions at stalled replication forks are still unclear, and the contribution of various factors poorly defined. We investigate in particular the function of the AAA+ ATPase WRNIP1 that seems to contribute to replication fork stabilisation under replication stress conditions in a so far unclear manner.

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MECHANISMS OF GENOME MAINTENANCE



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DNA replication stress, a phenomenon defined as the slowing or stalling of replication fork progression, is a major source of genomic instability in early stages of tumorigenesis and it is also a common feature of cancer cells. We are interested in understanding how replication stress gives rise to DNA damage and how cells deal with this pathological condition to preserve genomic stability.

Role of co-transcriptional R-loops in DNA replication stress

Shruti Menon, Anca-Irina Mihai

DNA replication stress arises upon activation of oncogenes as a consequence of premature entry into S phase that results in aberrant firing of intragenic replication origins. This leads to collisions between the transcription and replication machineries, which can cause chromosome breakage and rearrangements. Recent studies have shown that transcription-replication collisions (TRCs) promote the formation of co-transcriptional R-loops, a potent block to replication fork progression. However, whether or not R-loops are

the cause of oncogene-induced DNA damage remains elusive. We have established new methods for detection of R-loops in human cells. We could show that upon mild replication stress induced by a low dose of aphidicolin, R-loops formed at late-replicating common fragile sites (CFSs), while upon replication arrest induced by hydroxyurea, R-loops formed in highly transcribed genes harboring early replication fragile sites (ERFSs). Genome-wide analysis indicated that the sites of R-loop formation upon replication stress coincide with the regions that are recurrently deleted in human cancers. We are now extending our study to mapping R-loop-forming loci in cells with oncogene-induced replication stress. We would also like to pursue mass spectroscopy analysis of R-loop-containing chromatin fragments to identify new proteins involved in R-loop metabolism under conditions of replication stress.

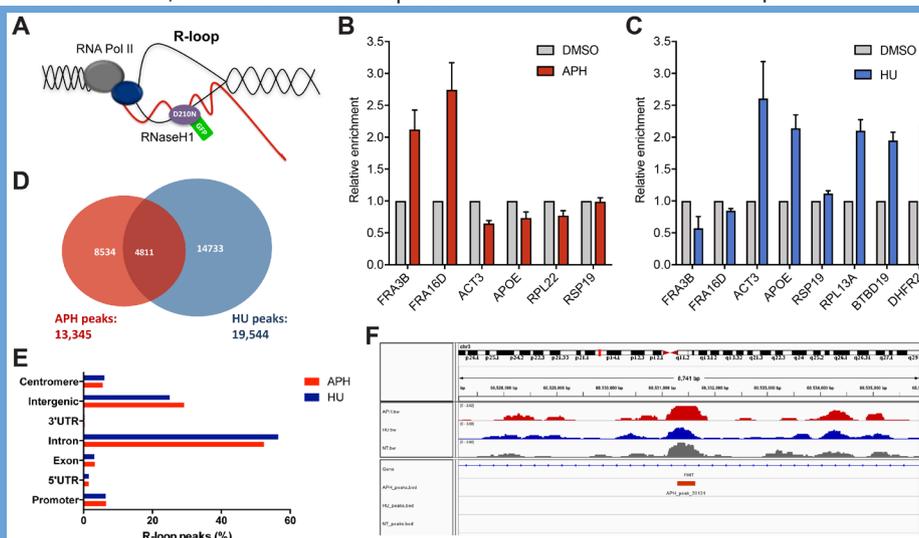


Figure 1. Formation of R-loops in response to DNA replication stress.

(A) Schematic depicting the tool used to detect R-loops: a fusion of green fluorescence protein (GFP) with an RNase H1 (RNH1) mutant harboring a D210N substitution in the catalytic domain, which binds to but does not degrade RNA:DNA hybrids. (B) Relative occupancy of RNH1(D210N)-GFP at common fragile sites (CFSs) and actively transcribed genes under conditions of mild replication stress. U2OS T-REX cells inducibly-expressing RNH1(D210N)-GFP were treated either with DMSO (mock) or 0.2 μ M aphidicolin (APH) for 16 hours. After pre-extraction, cells were fixed with formaldehyde and isolated chromatin was subjected to immunoprecipitation using anti-GFP antibody followed by qPCR analysis. (C) Relative occupancy of RNH1(D210N)-GFP at CFSs and actively transcribed genes upon replication arrest. Cells were treated with DMSO (mock) or 10 mM hydroxyurea (HU) for 6 hours. ChIP-qPCR analysis was performed as in (B). (D) Venn-diagram showing the number of APH- and HU-specific bindings sites of RNH1(D210N)-GFP as determined by ChIP-sequencing. U2OS T-REX cells inducibly-expressing RNH1(D210N)-GFP were treated with APH and HU as in (B) and (C), respectively. Peak calling was performed using MACS2, with DMSO sample as background. (E) Distribution of the RNH1(D210N)-GFP peaks represented in (D) across defined genomic features. (F) Integrated Genome Viewer (IGV) display of RNH1(D210N)-GFP occupancy along the region of the FHIT gene harboring the common fragile site FRA3B, for APH-treated, HU-treated and non-treated (NT, DMSO) cells.

Resolution of conflict between transcription and replication during S-phase

Nagaraja Chappidi, Esin Isik, Andreas Sturzenegger, Domino Schlegel

Although there is a great deal of knowledge about the strategies cells evolved to overcome TRCs or to eliminate R-loops, understanding of whether and how a replication fork blocked by an R-loop can restart DNA synthesis remains elusive. Our studies have identified RECQ5 DNA helicase as a genome maintenance factor that promotes the progression of replication forks through actively transcribed genes and prevents accumulation of RAD51 recombinase at sites of transcription-replication interference, suggesting a role for RECQ5 in TRC resolution. More recent work in our laboratory revealed that replication fork slowing caused by co-transcriptional R-loops is an active process that involves activation of the Fanconi anemia pathway leading to replication fork protection by RAD51 filaments, which is associated with replication fork reversal. Furthermore, we have found that the restart of replication forks stalled by R-loops requires active transcription, MUS81/EME1 endonuclease, RAD52 strand-annealing protein, the DNA ligase 4/XRCC4 complex and the non-catalytic subunit of DNA polymerase δ , POLD3. The switch from fork protection to replication restart is mediated by the DNA helicases RECQ1 and RECQ5 that counteract replication fork reversal and RAD51 filament assembly, respectively, thereby promoting fork cleavage by MUS81/EME1. We propose that this relieves the topological constraints generated by converging transcription and replication machineries, allowing reactivation of transcription and subsequent replication restart upon fork re-ligation.

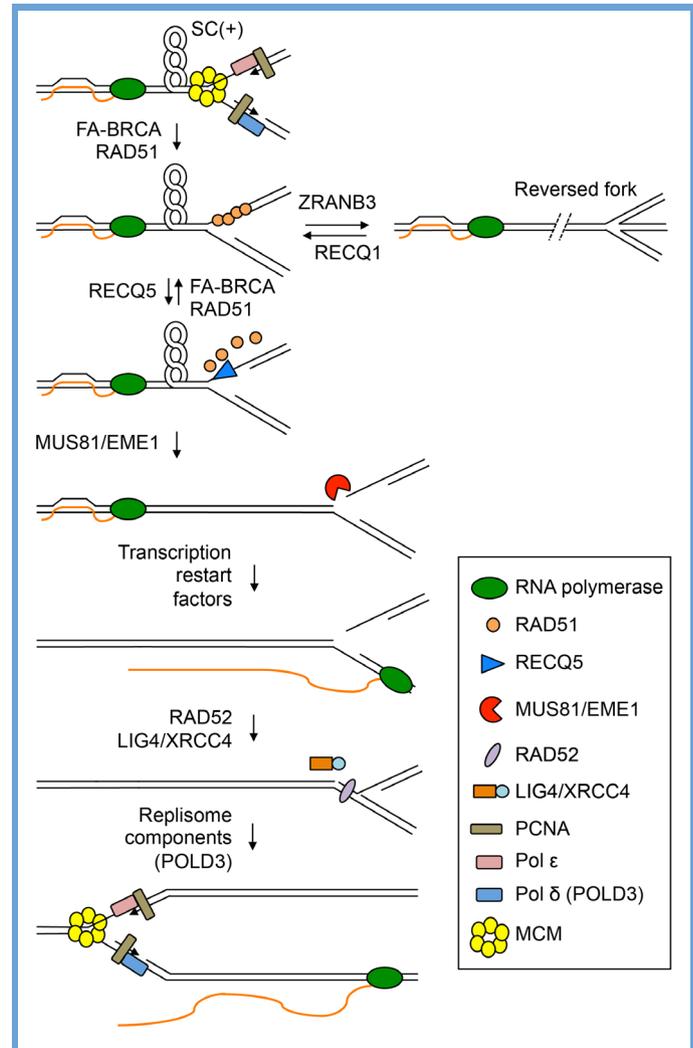


Figure 2. Model for resolution of transcription-replication collisions.

The blockage of replication forks by oncoming transcription complexes results from the build-up of positive supercoiling within the intervening DNA region and the formation of co-transcriptional R-loops. Replication fork stalling activates the FA pathway leading to the assembly of RAD51 filaments on the parental DNA strands at the fork junction, which promotes fork reversal by DNA translocases such as ZRANB3. RECQ1 DNA helicase counteracts replication fork reversal to promote TRC resolution. In this pathway, RECQ5 DNA helicase disrupts RAD51 filaments to facilitate fork cleavage by MUS81/EME1 endonuclease. This relieves the topological barrier in the DNA template, allowing transcription restart. After RNA polymerase passage, replication fork is re-stored by RAD52-mediated re-annealing of the parental strands and sealing the nick in the parental duplex by the LIG4/XRCC4 complex. This is followed by POLD3-dependent restart of semi-conservative DNA replication.

Mechanism of mitotic DNA synthesis

Stefano Di Marco, Naga Raja Chappidi, Shruti Menon

DNA replication has to be completed before the onset of anaphase to prevent chromosome mis-segregation and accumulation of DNA damage in newly born G1 cells, which can lead to chromosomal rearrangements. Recent studies have revealed that replication stress triggers DNA-repair synthesis in mitotic prophase, a phenomenon known as MiDAS that serves to complete replication of difficult-to-replicate loci such as CFSs. MiDAS depends on MUS81/EME1

endonuclease and is restrained by RAD51 and BRCA2. We have shown that CFS-associated MiDAS requires RECQ5 DNA helicase (Figure 3). Our work has revealed that RECQ5 is recruited to CFSs upon entry to mitosis through its interaction with MUS81. Moreover, we have shown that RECQ5 disrupts RAD51 nucleoprotein filaments stabilizing stalled replication forks at CFSs and hence facilitates MUS81-mediated fork cleavage, which triggers MiDAS. Our work also revealed that MiDAS depends on the formation of R-loops and requires active transcription, suggesting that it is triggered by R-loop-mediated TRCs and might be a part of the process leading to TRC resolution.

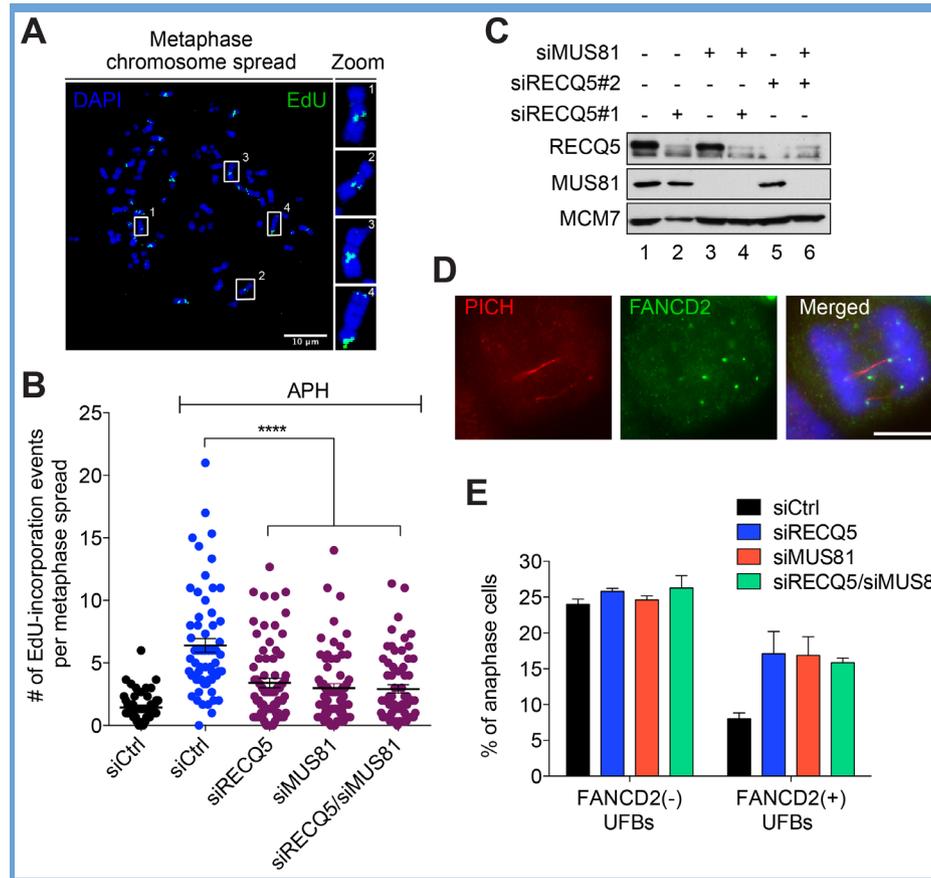


Figure 3. Role of RECQ5 DNA helicase in mitotic DNA synthesis.

(A) Visualization of sites of mitotic DNA synthesis on metaphase chromosomes. U2OS cells were treated with APH (0.4 μM) and RO-3306 (9 μM) for 16 h (late G2 arrest), and then released into fresh medium containing EdU (20 μM) and Colcemid (0.1 μg/ml) for a further 60 min. (B) Quantification of EdU incorporation on metaphase chromosomes for mock-, RECQ5-, MUS81- and MUS81/RECQ5-depleted cells treated as in (A).

(C) Western blot analysis of extracts of U2OS cells transfected with indicated siRNAs.

(D) Example of CFS-associated ultrafine bridges (UFBs) scored in RECQ5-depleted U2OS cells treated with 0.4 μM APH for 24 h. CFS-associated UFBs are marked by PIC1H on the bridge (red) and FANCD2 foci (green) on the bridge termini.

(E) Quantification of the frequency of centromeric (FANCD2-negative) and CFS-associated (FANCD2-positive) UFBs in U2OS cells transfected with indicated siRNA.

Funding

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JOSEF JIRICNY



THE MULTIFACETED MISMATCH REPAIR



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During the past four decades, my group has been primarily interested in studying the biochemistry and biology of the postreplicative mismatch repair (MMR) system in human cells. As mutations in MMR genes are associated with hereditary non-polyposis colon cancer (HNPCC, also known as Lynch Syndrome), one of the most common inherited cancer predisposition syndromes, our primary goal was to understand how MMR functions and how its malfunction leads to malignant transformation. However, recent evidence implicated MMR proteins also in other pathways of DNA metabolism, ranging from DNA damage signalling, through chromatin packaging and interstrand cross-link repair to antibody diversification. We are studying the interplay of these processes in human cells, and have used the CRISPR-Cas9 technology to generate single, double and triple knock-out cell lines to test our hypotheses. However, we are also exploring the potential of other biological systems, such as the DT40 chicken bursal B cells that are amenable to ready genetic manipulation. We are also revisiting the field of DNA methylation/demethylation, with a specific focus on the role of hormone receptors in the control of transcription.

The MMR Interactome I

Julia Pizzolato, Antonio Porro

FAN1 was identified as a strong interactor of the MMR protein MLH1. We could show that FAN1 is an exo/endonuclease, which preferentially cleaves 5' flaps and D-loops *in vitro*. We also showed that it is involved in the processing of interstrand cross-links (ICLs), as witnessed by the hypersensitivity of FAN1-deficient cells to cisplatin and mitomycin C (MMC). Interestingly, although FAN1 interacts with proteins of the FA ICL repair pathway, its deficiency does not cause Fanconi anemia. Instead, mutation carriers are afflicted with karyomegalic interstitial nephritis (KIN). In an attempt to throw some light on the biological role of FAN1, we set out to study its biochemical properties in greater detail. Using a synthetic oligonucleotide substrate containing an ICL reminiscent of a nitrogen mustard-like cross-link, we could show that FAN1 can traverse this lesion in both branched and linear substrates [Pizzolato, J., Mukherjee, S., Schärer, O.D. and Jiricny, J. (2015) *J. Biol. Chem.*, 290, 22602-11]. This unusual mode of action sets it apart from other ICL-processing nucleases, which act predominantly at arrested replication forks. The possible relevance of this finding to KIN remains to be elucidated.

We have also been studying the response of FAN1-deficient and overexpressing cells to a variety of reagents that interact with or modify DNA. Our data indicate that substances able to stabilize tertiary structures in DNA elicit a unique response in FAN1-overexpressing cells, which culminates in extensive post-translational modification of PCNA, the processivity factor of replicative DNA polymerases. In this study, we were able to show that FAN1 is recruited to stabilised G-quartet structures independently of FANCD2, which had hitherto been believed to be the upstream controller of FAN1 function. Surprisingly, recruitment to the secondary structures in DNA was dependent on mono-ubiquitylated PCNA, which interacts directly with FAN1 via a non-canonical PIP motif. [Porro, A., Berti, M., Pizzolato, J., Bologna, S., Kaden, S., Saxer, A., Ma, Y., Nagasawa, K., Sartori, A. A. and Jiricny J. (2017) *Nature Communications*, 8:1073]

The MMR Interactome II

Saho Kobayashi, Katja Kratz, Mariela Artola-Borán

MMR is highly-conserved in evolution, but, surprisingly, the eukaryotic pathway appears to be dependent on only a single exonuclease, EXO1, in contrast to prokaryotic MMR that utilises at least four nucleases. Because the mutator phenotype of MSH2- or MLH1-deficient cells is considerably stronger than that of cells lacking EXO1, additional nucleases were predicted to compensate (at least partially) for the lack of EXO1, but none could be identified to date. In our previous work, we studied the contribution of the proofreading activity of polymerase- δ to MMR. [Ghodgaonkar, M.M., Kehl, P., Ventura, I., Hu, L., Bignami, M. and Jiricny, J. (2015) *Nature Communications*, 5:4990]. More recently, we have studied the ability of FAN1 to compensate for EXO1 deficiency. We could show that FAN1 can indeed substitute for EXO1 in EXO1-depleted cell extracts, and in extracts of EXO1- or EXO1/FAN1 knock-out cells. We could also show that the efficiency of correction of a mismatch-carrying plasmid was lower in cells lacking both EXO1 and FAN1 than in cells disrupted in only one of these loci, or in wild-type cells. This implies that EXO1 and FAN1 are at least partially redundant. We could also show that a FAN1 variant mutated in the MLH1 interaction site is substantially less able to compensate for the lack of EXO1.

The MMR Interactome III

Sara Giovannini, Medini Ghodgaonkar, Svenja Kaden, Anja Saxer, Kalpana Surendranath, Marie-Christine Weller

In a yeast genetic screen, we identified a number of loci that appear to modulate the response of cells to methylating agents in a manner dependent on the integrity of the MMR system [Cejka, P., Fischer, F. and Jiricny, J. (2008) *Genetics*, 179, 1835-1844]. One of these was ELG1, which encodes an alternative large subunit of the heteropentameric Replication Factor C (RFC). In the yeast system, the ELG1/RFC2-5 complex was shown to unload PCNA from DNA, and the malfunction of this factor was linked to genomic instability. (ELG1 stands for Elevated Levels of Genomic instability.)

In an attempt to learn whether the human ortholog, ATAD5, fulfills a similar function, we have generated a stable cell line, in which we can inducibly knock-down ATAD5 with shRNA. Although we have to date failed to identify gross chromosomal abnormalities linked to ATAD5 loss in these cells, we detected an increase in sensitivity to several genotoxic agents, as well as a defect in the repair of single-strand DNA breaks. We are currently attempting to understand the molecular basis of this phenotype.

We have also attempted to express the ATAD5/RFC2-5 protein in the baculovirus expression system, albeit without success to date. Our efforts are continuing.

Molecular Mechanisms of Active DNA Demethylation

Lia Kallenberger, Rachel Erb

Hormone-activated nuclear receptors are potent transcriptional activators. In a report published a number of years ago [Saluz, H.-P.; Jiricny, J.; Jost, J.-P.; (1986) *Proc. Natl. Acad. Sci. USA*, 83, 7167-7171], the estrogen receptor (ER) was reported to activate a chicken oviduct gene, vitellogenin (VTG), in rooster, where the gene was silenced by DNA cytosine methylation. The transcriptional activation was accompanied by active DNA demethylation, as documented by genomic sequencing. Given that the proteins involved in active demethylation were not known at that time, we wanted to learn whether the estrogen-dependent transcriptional activation and demethylation involved any of the enzymes that have recently been linked to oxidation of 5-methylcytosine – the Ten-Eleven Translocation proteins 1-3 (TET1-3) – and Thymine DNA Glycosylase (TDG), believed to remove the oxidation products 5-formylcytosine and 5-carboxycytosine from DNA. We have generated a cell-based reporter system in which the VTG enhancer/promoter drives the expression of a luciferase gene. Our results to date suggest that, contrary to expectation, methylation of the estrogen-responsive element (ERE) in the enhancer has no effect on expression of the reporter. Moreover, estrogen treatment does not cause ERE demethylation. However, methylation of a VTG promoter element silences the gene. We are currently attempting to identify the methylation-responsive transcription factor(s) that binds to this element by mass spectrometry.

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MASSIMO LOPES



DNA REPLICATION STRESS IN CANCER AND STEM CELLS



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* left 2016/17

Our research focuses on the molecular characterization of DNA replication stress and its contribution to genome instability. We aim to understand the mechanistic basis of genome rearrangements arising during perturbed DNA replication in stem and somatic cells, affecting various aspects of human disease and most specifically cancer. These studies take advantage of an established technological platform, ranging from standard molecular and cell biology methods to specialized single-molecule *in vivo* analysis of replication intermediates.

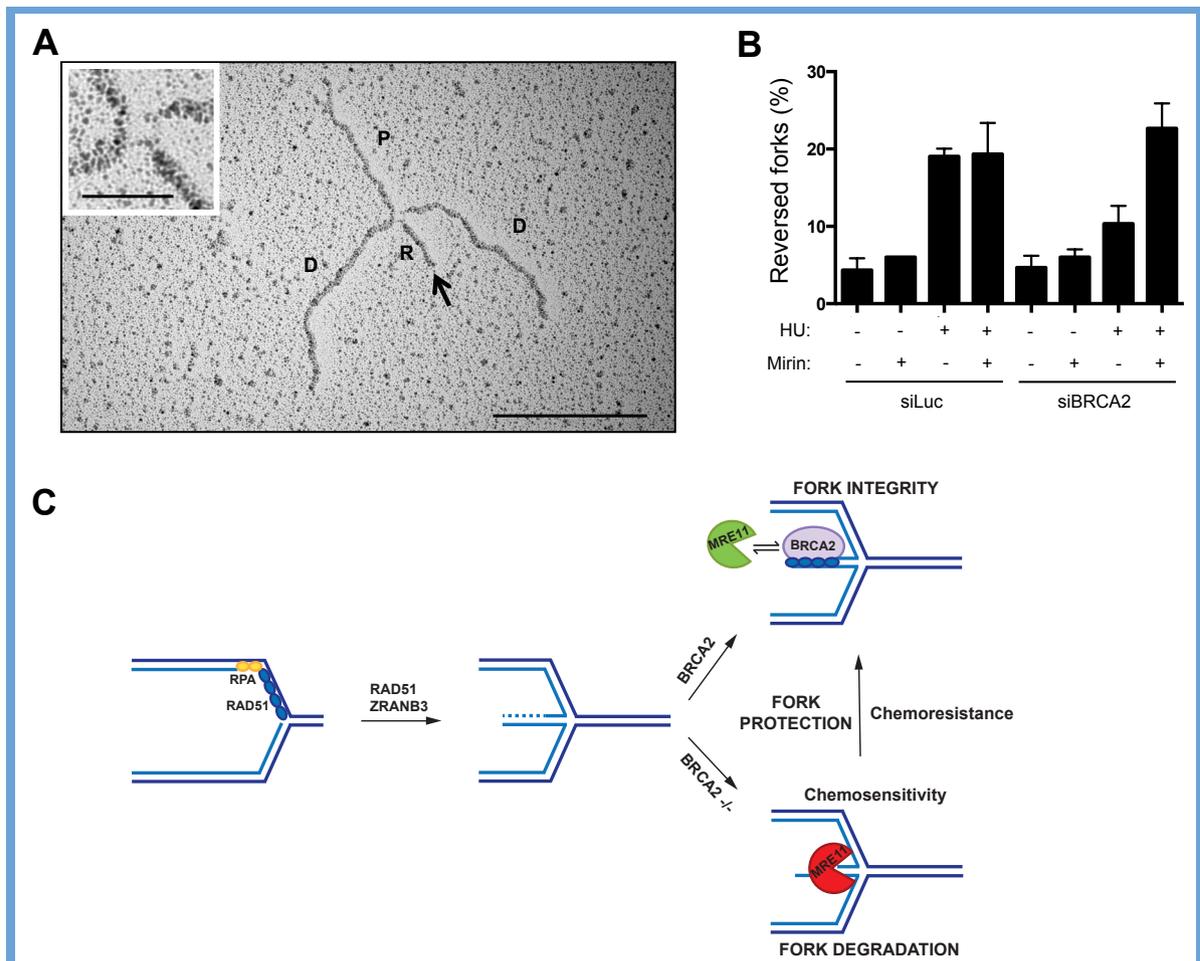
Replication fork remodeling upon cancer chemotherapeutic treatments

Ralph Zellweger, Marko Vujanovic, Sofija Mijic, Karun Mutreja, Matteo Berti, Jana Krietsch, Sebastian Ursich, Maria Dilia Palumbieri

DNA replication interference is one of the most common strategies employed in cancer chemotherapy. Although many of these drugs have been used in the clinics for decades, their molecular mechanism of action is often poorly understood, preventing the informed selection of appropriate chemotherapeutic regimens for different tumors and the development of potent combinatorial treatments. Combining classical cell and molecular biology with specialized single-molecule approaches on replication intermediates (Zellweger and Lopes, *Meth Mol Biol* 2018), we have uncovered surprising alterations of replication fork architecture upon several chemotherapeutic treatments. Most notably we reported the conversion of a high number of replication forks into four way junctions (Figure 1A), a process also known as replication fork reversal (Ray Chaudhuri et al., *Nature Str Mol Biol* 2012; Zellweger et al., *JCB* 2015; Neelsen and Lopes, *Nature Rev Mol Cell Biol* 2015). We have provided several lines of evidence that these transactions at replication forks are transient and genome protective, making them attractive targets for combinatorial chemotherapy. We have thus recently started to elucidate the cellular factors playing a role in the formation and resolution of these structures, and uncovered the central recombinase RAD51, PCNA polyubiquitination and the DNA translocase ZRANB3 as strictly required for drug induced fork reversal (Zellweger et al., *JCB* 2015; Vujanovic et al., *Mol Cell* 2017). Despite their genome maintenance function, we have shown that reversed forks can also become entry points for extensive, nuclease-mediated degradation of newly replicated DNA (Mijic et al., *Nature Comms* 2017), which was recently reported as a crucial molecular determinant of the exquisite sensitivity to cancer chemotherapeutics observed in BRCA-defective tumors (Figure 1B-C).

Most recently we have investigated the peculiar transactions that allow replication forks to bypass and repair inter-strand crosslinks. These are particularly toxic adducts – induced by byproducts of cell metabolism, as well as several chemotherapeutic drugs – which were long considered roadblocks to the replication process, but are in fact mysteriously bypassed at high efficiency during genome replication of human cells (Mutreja et al., submitted). Given the crucial role of replication fork remodeling in the response to chemotherapeutic treatments, we are now attempting to build specific

read-outs for these molecular transactions, in order to use them for genome-wide screens and to reveal novel cellular factors modulating chemo-sensitivity or chemo-resistance in specific types of cancer. We also plan to expand these studies from cancer cell lines into animal models and clinical samples, in order to exploit these specialized approaches as prognostic tools, prospectively contributing to cancer personalized medicine.



DNA replication stress in stem cells

Kurt Jacobs, Jana Krietsch, Sebastian Ursich

Embryonic stem cells (ESCs) have the unique ability to self-renew and are capable of differentiating into multiple cell types. In contrast, adult stem cells - such as hematopoietic stem cells (HSCs) - repopulate specific tissues. The exhaustion of adult stem cells has been linked to ageing, but the underlying molecular mechanisms are still largely unknown. Several knockout-mouse models have uncovered a role for numerous DNA repair factors in ageing and cancer. Besides well-known repair activities, conditional deletion of the ATR gene - which is a central factor activated in response to DNA replication stress - causes depletion of the stem cell niche, suggesting that stem cells need to protect their genomes during active proliferation. We are investigating the intriguing connection between replication stress and aging, applying some of our most revealing approaches to different populations of stem cells. We have recently

shown in cultured ESCs and mouse embryos that H2AX phosphorylation is dependent on ATR and is associated with chromatin loading of the ssDNA-binding proteins RPA and RAD51 (Figure 2A; Ahuja et al., *Nature Comms* 2016). Single-molecule analysis of replication intermediates reveals massive ssDNA gap accumulation, reduced fork speed and frequent fork reversal. All these marks of replication stress - which surprisingly do not impair the mitotic process - are rapidly lost at the onset of differentiation and result from the rapid transition through the G1 phase, which is strictly required to maintain pluripotency. In this context, when cell cycle checkpoints are mostly inactive and numerous DNA lesions are channeled into replication, fork slowing and reversal are strictly required to avoid chromosomal breakage and represent an effective alternative strategy of genome maintenance, compatible with the high proliferation rate of these cells (Figure 2B; Ahuja et al. *Nature Comms* 2016). In light of the recently proposed links between adult stem cell proliferation, DNA damage and cancer, we are now expanding these studies to investigate whether activation of quiescent adult stem cells leads to detectable replication stress phenotypes.

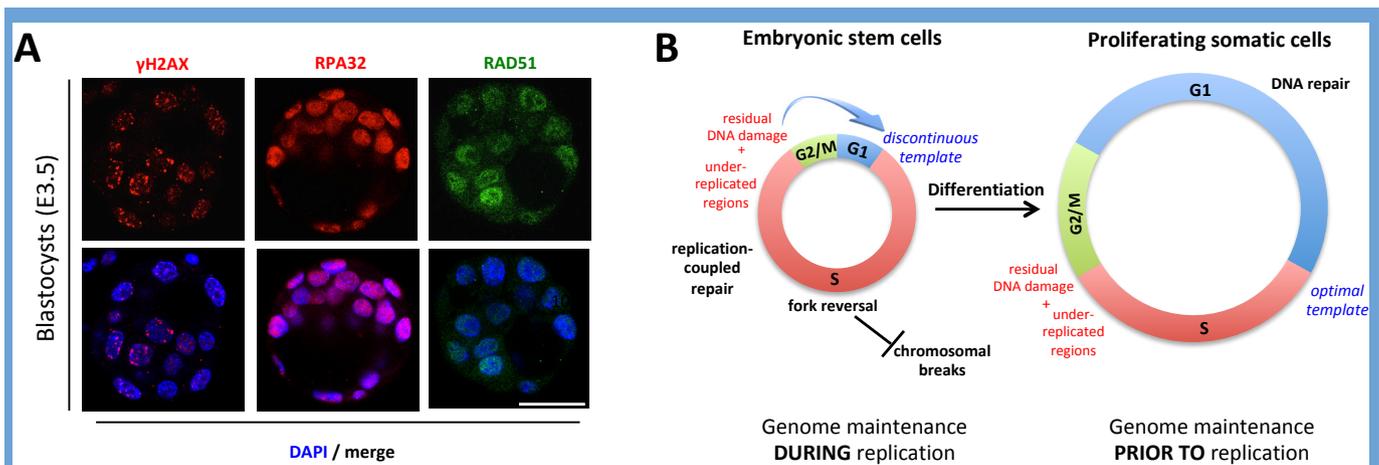


Figure 2. Replication stress markers in embryonic stem cells and their alternative, replication-coupled strategy for genome maintenance (from Ahuja et al. *Nature Comms* 2016).

(A) Immunofluorescence staining for the DNA damage-marker γ H2AX, and for chromatin binding of the ssDNA-binding proteins RPA32 and RAD51 in E3.5 blastocysts. The results indicate activation of DNA damage response and accumulation of ssDNA in unperturbed mouse embryonic stem cells (ESCs) within their natural environment.

(B) A model depicting differential control of genome stability in ESCs and proliferating somatic cells. Under-replicated regions and residual DNA damage are unavoidably present at the end of each S phase in both ESCs and somatic cells. Differentiated cells have prolonged gap phases and repair most of these lesions prior to S phase entry. Conversely, owing to the brief gap phases, ESCs channel a high number of these lesions into the following S phase and protect genome integrity by extensive fork reversal and replication-coupled repair.

Replication stress during unperturbed S-phase and upon oncogene activation

Katharina Zwicky, Sofija Mijic, Jonas Schmid, Henriette Stoy, Sebastian Ursich

Besides the established role of reversed forks in response to chemotherapeutic treatments (see above), we have provided solid evidence that these unusual intermediates accumulate also during unperturbed S phase, at regions that are intrinsically difficult to replicate, such as repetitive sequences (Follonier et al., *NSMB* 2013; Ray Chaudhuri et al., *MCB* 2015). Intriguingly, in light of the remarkable similarity of regressed arms to double-stranded breaks (DSB), we have uncovered – in collaboration with the Penengo lab here at the IMCR – a crucial role for classical DSB-response factors in promoting reversed fork stability and restart, mediating efficient fork progression during unperturbed S phase (Schmid et al., submitted; see “Lorenza Penengo” group). We are also currently investigating another type of endogenous replication stress, linked to replication-transcription interference and to accumulation of DNA-RNA hybrids, known as R-loops. We are attempting to provide direct visualization of these events in human cells and to understand the molecular mechanism of interference that these events are known to exert on the replication process.

We have also contributed to uncover the molecular determinants of oncogene-induced replication stress, which is known as one of the earliest causative events in tumorigenesis. We have applied our specialized investigation platform to cell culture models, studying specific genetic alterations frequently associated with early tumorigenesis, such as CyclinE- and CDC25A overexpression, or partial DNA re-replication (Neelsen et al., *JCB* 2013; Neelsen et al., *G&D* 2013). We are currently extending our studies to additional oncogenes and to more clinically relevant experimental systems, including 3D cultures of cancer cells and simultaneous alterations of oncogenes and tumor suppressors. We aim to investigate how multiple alterations can concur to fuel genomic instability in early tumorigenesis, uncovering the specific contributions of R-loops and replication/transcription interference in these phenomena.

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GIANCARLO MARRA



COLON CANCER



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One of the best known processes of tumorigenesis in humans is that which occurs in the colon (or large intestine). Thanks to major advances achieved in the last three decades in the fields of endoscopy, histology and molecular pathology, cancer of the large intestine is no longer viewed as a single disease entity: several distinct phenotypes have been identified, and this phenotypic variability is already evident in the precancerous lesions that develop in the gut mucosa.

Even today, precancerous lesions are often referred to collectively as colorectal polyps. However, although most of these premalignant lesions are raised, polyp-like growths, more recent research has revealed that there are others that are only slightly elevated above the mucosal surface, flat, or even depressed like a crater. Polyps are much easier to see during routine colonoscopy, and that is one reason they have received so much attention. But the nonpolypoid lesions are now being identified with increasing frequency, in part because clinicians are becoming more aware of their existence and importance, and in part because of the development of more sensitive endoscopic techniques.

Precancerous colorectal lesions are also collectively referred to as adenomas. This term refers to the pattern of cellular dysplasia seen by the pathologist who examines the lesion under a microscope. The adenomatous pattern is very common in precancerous colorectal lesions, but it is not the only pattern. Some benign lesions have cells that are arranged in a saw-toothed or serrated pattern, and they seem to give rise to a particular colorectal cancer phenotype.

The phenotype of a tumor is the outward expression of the specific genetic and epigenetic alterations found in the tumor cells. Some of these somatic alterations have already been well defined; others have been partially characterized, and many have yet to be identified. Changes affecting the genes have wide-ranging effects that are not limited to the appearance of the tumor and the arrangement of its cells: they also determine the tumor behaviour, its aggressiveness and responsiveness to anti-cancer drugs. Thanks to the availability of high-throughput analytical tools (genomics, epigenomics, transcriptomics, proteomics, metabolomics etc.), we can now identify, in each colon tumor tissue, a vast number of molecular characteristics that produce these phenotypic features— and this is an essential step toward individualized (and hopefully more effective) treatment regimens.

Targeted proteomics in colorectal tumors

Anuli Uozie

Targeted proteomic methods can accelerate the verification of multiple tumor marker candidates in large series of patient samples. We utilized the targeted approach known as selected/multiple reaction monitoring (S/MRM) to verify potential protein markers of colorectal adenoma identified by our group in previous transcriptomic and quantitative shotgun proteomic studies of a large cohort of precancerous colorectal lesions. We developed SRM assays to reproducibly detect and quantify 25 (62.5%) of the 40 selected proteins in an independent series of precancerous and cancerous tissue samples (19 adenoma/normal mucosa pairs; 17 adenocarcinoma/normal mucosa pairs). Twenty-three proteins were significantly up-regulated ($n = 17$) or downregulated ($n = 6$) in adenomas and/or adenocarcinomas, as compared with normal mucosa (linear fold changes $\geq \pm 1.3$, adjusted P value < 0.05). Most changes were observed in both tumor types (up-regulation of ANP32A, ANXA3, SORD, LDHA, LCN2, NCL, S100A11, SERPINB5, CDV3, OLFM4, and REG4; downregulation of ARF6 and PGM5), and a five-protein biomarker signature distinguished neoplastic tissue from normal mucosa with a maximum area under the receiver operating curve greater than 0.83. Other changes were specific for adenomas (PPA1 and PPA2 up-regulation; KCTD12 downregulation) or adenocarcinoma (ANP32B, G6PD, RCN1, and SET up-regulation; downregulated AKR1B1, APEX1, and PPA1) (Figure 1). Some changes significantly correlated with a few patient- or tumor-related phenotypes. Twenty-two (96%) of the 23 proteins have a potential to be released from the tumors into the bloodstream, and their detectability in plasma has been previously reported. The proteins identified in this study expand the pool of biomarker candidates that can be used to develop a standardized pre-colonoscopy blood test for the early detection of colorectal tumors.

Tissue samples collected at the Gastroenterology Unit of the Hospital of Cremona, Italy (Prof. Federico Buffoli).

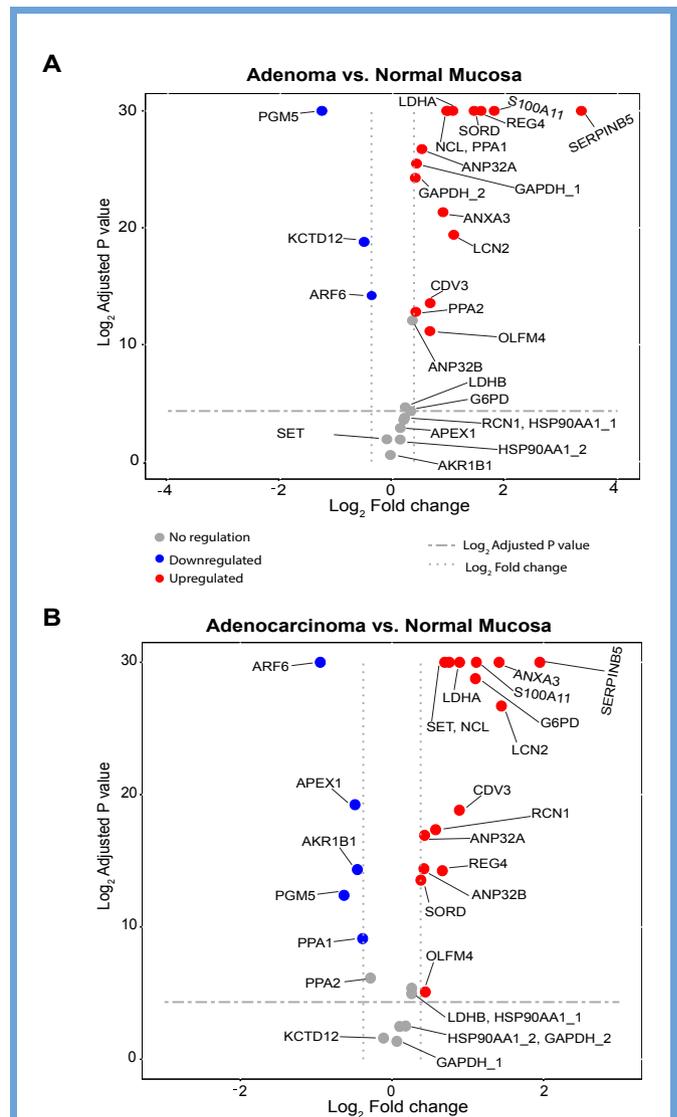


Figure 1. Quantification of protein tumor markers in colorectal adenomas (A) and adenocarcinomas (B) (relative to matched normal mucosal samples).

Proteins with an adjusted P value < 0.05 and a fold change $\geq \pm 1.3$ were considered significant. Housekeeping proteins are denoted with ₁ and ₂.

The metabolome and lipidome of colorectal tumors

David J. Fischer

In this study, we aimed to characterize the metabolome (metabolites and lipids) of colorectal adenomas, in comparison with that of colorectal cancers.

The series comprised 39 colorectal tumors: 29 adenomas (pre-cancerous lesions) and 10 adenocarcinomas from 31 patients. For each tumor, a paired sample of normal mucosa from the same colon segment (>2 cm from the lesion) was collected. Therefore, a total of 78 tissue samples were analyzed for their metabolomes. We used capLCMS for the untargeted analysis of normal and tumor (both adenomas and adenocarcinomas) tissue metabolomes and lipidomes. Two chromatographic setups, HILIC (Hydrophilic Interaction Liquid Chromatography) and RP (reversed phase), were chosen to measure a broad range of both polar and non-polar compounds in these tissues. Since non-polar compounds include lipids, their complement in our tissues is termed *lipidome*, whereas that of HILIC-detected analytes is termed *metabolome*. Processing of raw data yielded a total of 1830 metabolites and 2365 lipids. The procedure of compound identification resulted in 760 metabolites and 1028 lipids with unique annotation. To find correlations between omics data profiles and clinical phenotypes, different levels of each phenotype were considered in the Between Group Analysis using the full set of detected metabolites and lipids. The phenotype “tissue type” revealed the highest number of statistically significant, differentially regulated compounds within the metabolome or the lipidome: the three levels of this phenotype (normal mucosa samples, adenomas or cancers) have markedly different metabolomic and lipidomic profiles (Figure 2). The first ordination axis invariably separated normal mucosa samples from tumors (adenomas and adenocarcinomas), indicating that the largest amount of omics changes accounts for the difference between normal mucosa and tumors. The second axis separated the two tumor types, adenomas from adenocarcinomas. The adenocarcinoma cluster was further apart from the normal mucosa cluster in both datasets, anticipating that omics changes common to the two types of tumors would be more pronounced in cancers. In addition, the metabolome of the normal-appearing colorectal mucosa of patients with adenocarcinomas appears slightly different from that of the normal mucosa of patients with adenomas.

Several metabolic pathways were similarly dysregulated in adenomas and adenocarcinomas, but specific metabolic alterations were also found in each tumorigenic stage (work in progress).

Tissue samples collected at the Gastroenterology Unit of the Zurich University Hospital (Dr. Christine Manser).

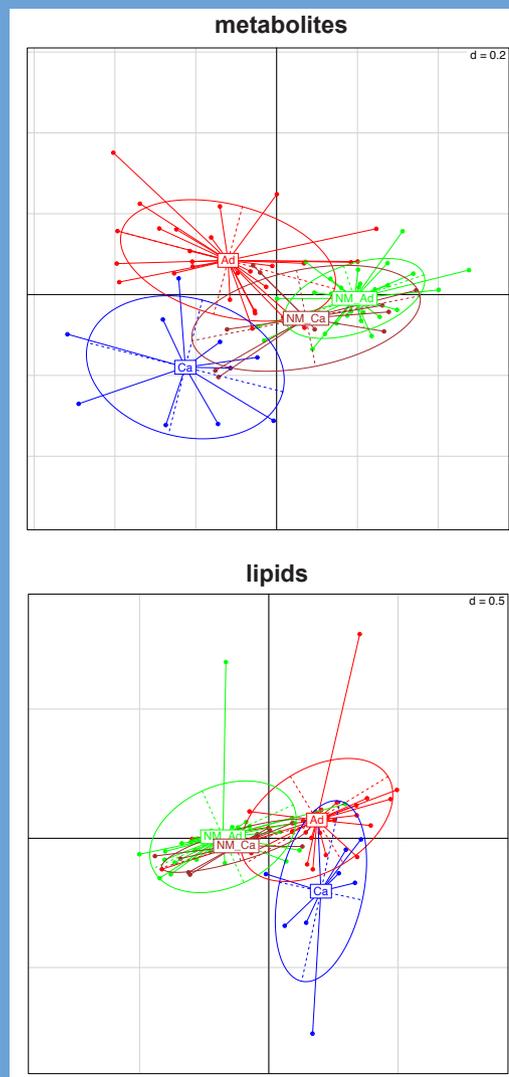


Figure 2. Between Group Analysis (BGA) of metabolomic (upper panel) and lipidomic (lower panel) data. The BGA was calculated using the phenotype “tissue type” with the levels: normal mucosa (NM_Ad, normal mucosa of patients with adenoma, NM_Ca, normal mucosa of patients with carcinoma), adenomas (Ad) and cancers (Ca). The two panels show the score plot (samples) of the first two BGA axes. The group center for each group is indicated by a rectangle and a 50% confidence ellipse is shown for each group.

The immune landscapes of polypoid and nonpolypoid precancerous colorectal lesions

Giancarlo Marra, in collaboration with Dr. Rosalia Maglietta (Institute of Intelligent Systems for Automation, National Research Council, Italy)

Little is known about the immunoediting process in precancerous lesions. We explored this aspect of benign colorectal adenomas with a descriptive analysis of the immune pathways and immune cells whose regulation is linked to the morphology and size of these lesions. Two series of polypoid and nonpolypoid colorectal adenomas were used in this study: 1) 84 samples (42 lesions, each with matched samples of normal mucosa) whose gene expression data were used to quantify the tumor morphology- and size-related dysregulation of immune pathways collected in the Molecular Signature Database, using Gene Set Enrichment Analysis; 2) 40 other lesions examined with immunohistochemistry to quantify the presence of immune cells in the stromal compartment. In the analysis of transcriptomic data, 429 immune pathways displayed significant differential regulation in neoplasms of different morphology and size. Most pathways were significantly upregulated or downregulated in polypoid lesions versus nonpolypoid lesions (regardless of size). Differential pathway regulation associated with lesion size was observed only in polypoid neoplasms. These findings were mirrored by tissue immunostaining with CD4, CD8, FOXP3, MHC-I, CD68, and CD163 antibodies: stromal immune cell counts (mainly T lymphocytes and macrophages) were significantly higher in polypoid lesions (Figure 3). Certain markers displayed significant size-related differences regardless of lesion morphology. Multivariate analysis of variance showed that the marker panel clearly discriminated between precancerous lesions of different morphologies and sizes. Statistical analysis of immunostained cell counts fully support the results of the transcriptomic data analysis: the density of infiltration of most immune cells in the stroma of polypoid precancerous lesions was significantly higher than that observed in nonpolypoid lesions. Large neoplasms also have more immune cells in their stroma than small lesions. Immunoediting in precancerous colorectal tumors may vary with lesion morphology and stage of development, and this variability could influence a given lesion's trajectory to cancer.

Tissue samples collected at the Gastroenterology Unit of the Hospital of Cremona, Italy (Prof. Federico Buffoli).

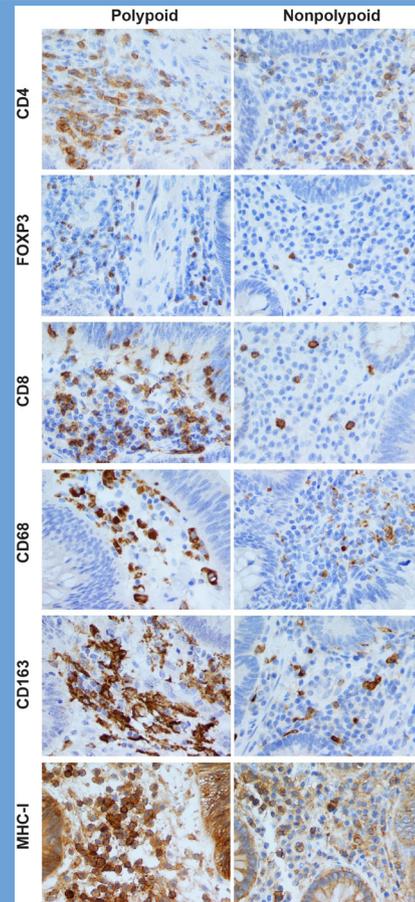


Figure 3. Immunohistochemical assessment of stromal immune cell infiltrates in polypoid and nonpolypoid colorectal adenomas. CD4+, FOXP3+, CD8+, CD68+, CD163+, and MHC-I+ immune cell densities (i.e., number of positively-stained cells per unit area) in the stroma of polypoid adenomas were consistently higher than those observed in nonpolypoid lesions. Intraepithelial CD8+ (cytotoxic) T cells are also seen in the epithelial compartments of both types of tumor. In calculating CD68+ and CD163+ macrophage densities, we counted only nuclei surrounded by CD68+ or CD163+ granulations and excluded elongated or fragmented cells with granular cytoplasmic positivity. MHC-I labelling (granular, compact, or membranous) was observed in a variety of stromal cells. MHC-I+ epithelial cells were also present in both types of tumor, but their presence was not quantified in this study. Magnification for all panels: 400X.

MSH3 mutations represent an additional recessive phenotype of colorectal adenomatous polyposis

Giancarlo Marra, in collaboration with Prof. Stefan Aretz (Institute of Human Genetics, University of Bonn, Germany)

In ~30% of families affected by colorectal adenomatous polyposis, no germline mutations have been identified in the previously implicated genes *APC*, *MUTYH*, *POLE*, *POLD1*, and *NTHL1*, although a hereditary etiology is likely. To uncover further genes with high penetrance causative mutations, we performed exome sequencing of leukocyte DNA from 102 unrelated individuals with unexplained adenomatous polyposis. We identified two unrelated individuals (belonging to Families 1275 and 1661) with differing compound-heterozygous loss-of-function (LoF) germline mutations in the mismatch-repair gene *MSH3*. The impact of the *MSH3* mutations

(c.1148delA, c.2319 1G>A, c.2760delC, and c.3001 2A>C) was indicated at the RNA and protein levels. Analysis of the diseased individuals' tumor tissue demonstrated high microsatellite instability of di- and tetra-nucleotides, and immunohistochemical staining illustrated a complete loss of nuclear MSH3 in normal and tumor tissue (Figure 4), confirming the LoF effect and causal relevance of the mutations. The pedigrees, genotypes, and frequency of *MSH3* mutations in the general population are consistent with an autosomal-recessive mode of inheritance. Both index persons have an affected sibling carrying the same mutations. The tumor spectrum in these four persons comprised colorectal and duodenal adenomas, colorectal cancer, gastric cancer, and an early-onset astrocytoma. Additionally, we detected one unrelated individual with biallelic *PMS2* germline mutations, representing constitutional mismatch-repair deficiency. Potentially causative variants in 14 more candidate genes identified in 26 other individuals require further workup. In the present study, we identified biallelic germline *MSH3* mutations in individuals with a suspected hereditary tumor syndrome.

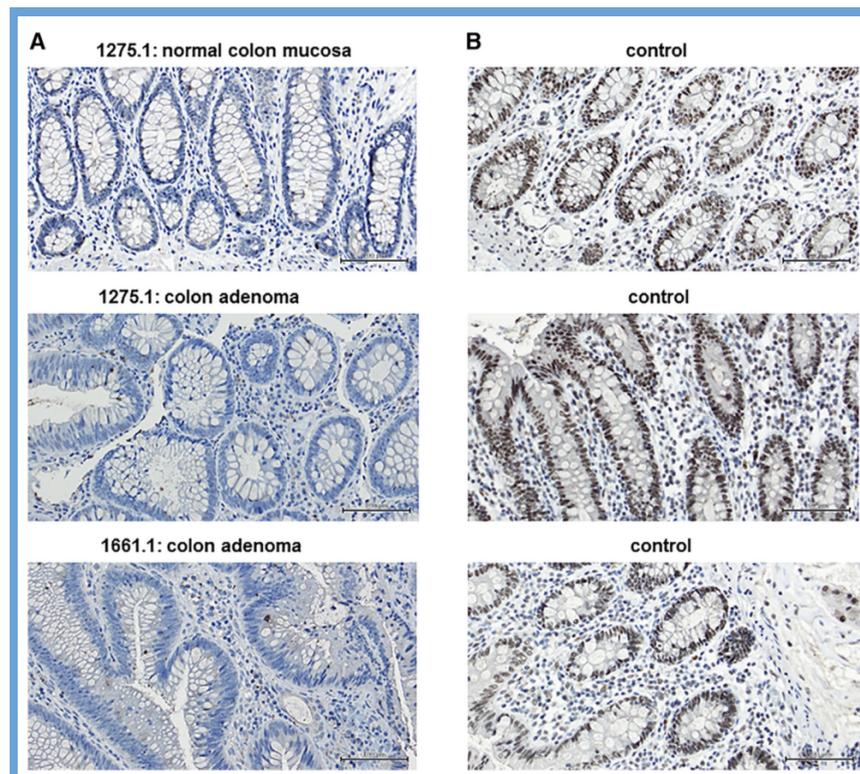


Figure 4. Immunostaining of MSH3 in Tumor and Normal Tissue.

MSH3 was stained with rabbit polyclonal antibody targeting N-terminal human MSH3 (antibody developed at IMCR, Univ. of Zurich).

(A) MSH3 was nearly undetectable in FFPE normal colon mucosa and colorectal adenoma samples from index person 1275.1 and a colorectal adenoma sample from index person 1661.1.

(B) In contrast, control samples, which were taken from FFPE normal mucosa of an independent subject with colon cancer and were processed in parallel, show a strong nuclear

Broccoli ingredient has positive influence on drug efficacy

Melanie M. Erzinger (Swiss National Foundation, SINERGIA project: Profs. Shana J. Sturla, Bernd Wollscheid, Niko Beerenwinkel and Giancarlo Marra)

The chemoprotective properties of sulforaphane (SF), derived from cruciferous vegetables, are widely acknowledged to arise from its potent induction of xenobiotic-metabolizing and antioxidant enzymes. However, much less is known about the impact of SF on the efficacy of cancer therapy through the modulation of drug-metabolizing enzymes. To identify proteins modulated by a low concentration of SF, we treated HT29 colon cancer cells with 2.5 μ M SF. Protein abundance changes were detected by stable isotope labeling of aminoacids in cell culture. Among 18 proteins found to be significantly up-regulated (Figure 5), aldo-ketoreductase 1C3 (AKR1C3), bioactivating the DNA cross-linking prodrug PR-104A, was further characterized. Preconditioning HT29 cells with SF reduced the EC50 of PR-104A 3.6-fold. The increase in PR-104A cytotoxicity was linked to AKR1C3 abundance and activity, both induced by SF in a dose-dependent manner. This effect was reproducible in a second colon cancer cell line, SW620, but not in other colon cancer cell lines where AKR1C3 abundance and activity were absent or barely detectable and could not be induced by SF. Interestingly, SF had no significant influence on PR-104A cytotoxicity in non-cancerous, immortalized human colonic epithelial cell lines expressing either low or high levels of AKR1C3. In conclusion, the enhanced response of PR-104A after preconditioning with SF was apparent only in cancer cells provided that AKR1C3 is expressed, while its expression in non-cancerous cells did not elicit such a response. Therefore, a subset of cancers may be susceptible to combined food-derived component and prodrug treatments with no harm to normal tissues.

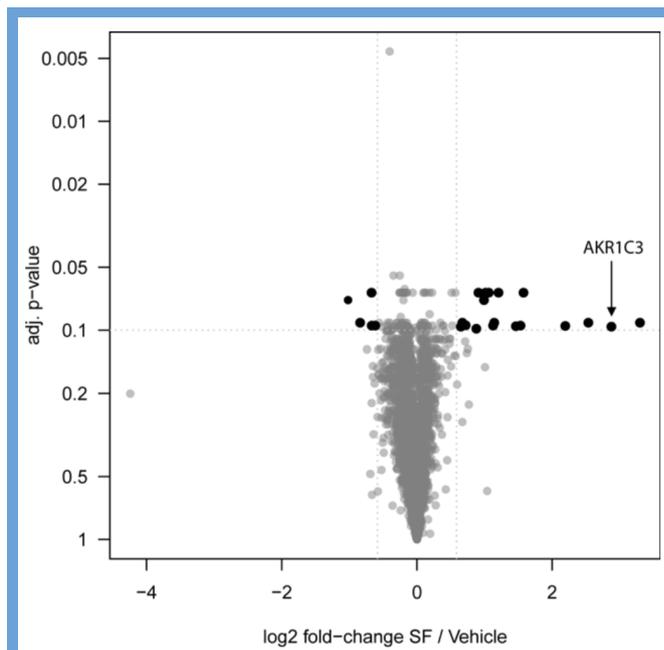


Figure 5. Volcano plot showing significant changes in the proteome following exposure of HT29 cells to 2.5 μ M SF for 48 h, determined by SILAC.

2653 proteins (black and gray) were quantified and tested for significance. Among the 23 significantly regulated proteins (black), 18 were up- and 5 down-regulated.

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HELICOBACTER PYLORI AND GASTRIC CANCER



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Our laboratory is interested in elucidating the mechanisms linking chronic inflammation to carcinogenesis. Our model system is the infectious agent *Helicobacter pylori*, which persistently colonizes the human stomach and causes gastritis, ulcers and gastric cancer. We use cell culture and animal models to better understand how the immune response to the infection, but also bacterial virulence factors and host factors such as gender and age at time of infection influence disease outcome.

Epigenetic control of diffuse large B-cell lymphoma

Hind Hashwah, Corina Schmid

Our lab has a long-standing interest in understanding the epigenetic and post-transcriptional mechanisms driving lymphomagenesis. In particular, we are interested in microRNAs and their deregulation, as well as the contribution of mutations in epigenetic modifiers to the pathogenesis of diffuse large B-cell lymphoma (DLBCL). The genes encoding the histone acetyl-transferases (HATs) CREBBP and EP300 are recurrently mutated in the activated B-cell-like and germinal center (GC) B-cell-like subtypes of DLBCL. To address the consequences of these mutations in various *in vitro* and *in vivo* models, we introduced a recurring patient mutation into a human DLBCL cell line using CRISPR and deleted Crebbp and Ep300 in the GC B-cell compartment of mice. CREBBP-mutant DLBCL clones exhibited reduced histone H3 acetylation, expressed significantly less MHCII and grew faster than wild type clones in subcutaneous and orthotopic xenograft models (Figure 1A-D). Mice lacking Crebbp in GC B-cells exhibited hyperproliferation of their GC compartment upon immunization, had reduced MHCII surface expression on GC cells, and developed accelerated MYC-driven lymphomas (Figure 1E,F). Ep300 inactivation reproduced some, but not all, consequences of Crebbp inactivation. MHCII deficiency phenocopied the effects of CREBBP loss in spontaneous and serial transplantation models of MYC-driven lymphomagenesis (Figure 1G), supporting the idea that the mutational inactivation of CREBBP promotes immune evasion. Indeed, the depletion of CD4⁺ T-cells greatly facilitated the engraftment of lymphoma cells in serial transplantation models. In summary, we provide evidence that both HATs are bona fide tumor suppressors that control MHCII expression and promote tumor immune control; mutational inactivation of CREBBP, but not of EP300, has additional cell-intrinsic engraftment and growth-promoting effects.

Hashwah, H., Schmid, C.A., Kasser, S., Bertram, K., Stelling, A., Manz, M.G., and Müller, A. Inactivation of CREBBP expands the germinal center B cell compartment, down-regulates MHCII expression and promotes DLBCL growth. PNAS, 114:9701-9706 (2017).

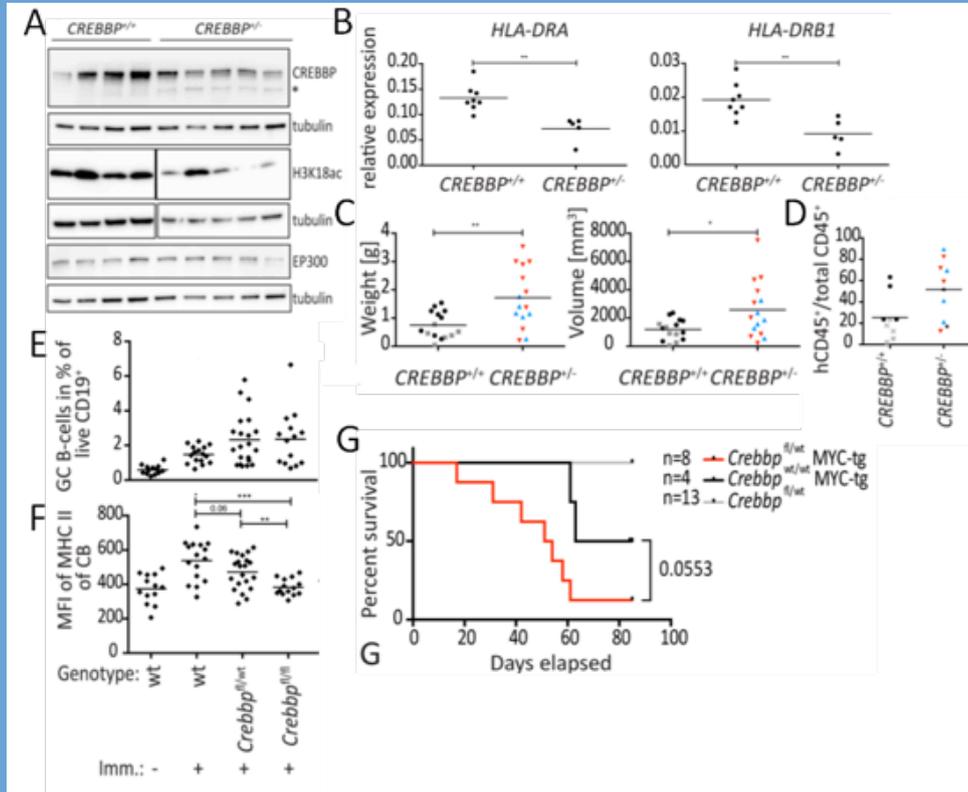


Figure 1. Inactivation of CREBBP reduces histone acetylation and HLA expression, and accelerates lymphoma growth in xenotransplantation and genetically modified mouse models.

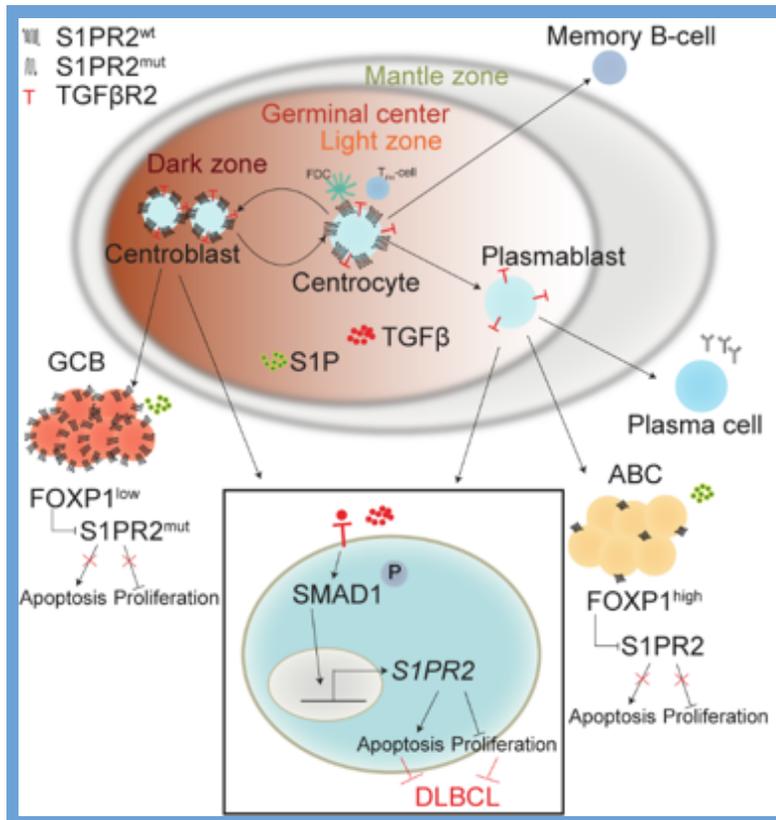
(A) Protein lysates from four CREBBP^{+/+} and five CREBBP^{-/-} clones were subjected to immunoblot analysis with antibodies specific for CREBBP, EP300, α -tubulin, and H3K18ac. (B) Relative expression of HLA-DRA and HLA-DRB1 compared to RPLP0 as assessed by qRT-PCR of the clones shown in B, plus several additional wild type clones. **p<0.01. (C) Ten million cells of two CREBBP^{+/+} and two CREBBP^{-/-} clones were subcutaneously transplanted onto the flanks of MISTRG mice. Tumor volumes and weights were determined at the study endpoint. Each symbol represents one tumor. Results from two pooled experiments are shown. (D) Ten million cells of three CREBBP^{+/+} and three CREBBP^{-/-} clones were intravenously transplanted into MISTRG mice; mice were sacrificed at four weeks post transplantation and assessed with respect to their human tumor burden in the bone marrow. (E) Littermates with either no (wt), one (fl/wt) or two (fl/fl) floxed alleles of *Crebbp*, which additionally express Cre under the control of the AID promoter, were immunized 4 times at biweekly intervals with sheep red blood cells and sacrificed 10 days after the last injection. Frequencies of CD95⁺CD38^{low} GC B-cells are shown in % of all CD19⁺ live cells. Non-immunized mice are included for comparison. Pooled data from three to five independent cohorts are shown. (F) Median fluorescence intensity (MFI) of MHCII surface expression on centroblasts of the mice shown in E. (G) Mice of the three indicated genotypes were immunized at regular 14 day intervals and monitored for clinical symptoms and enlarged lymph nodes indicating lymphoma development. Moribund mice were sacrificed and their survival time since the first immunization was plotted.

The TGF- β /SMAD1/S1PR2 signaling axis controls DLBCL growth *in vitro* and *in vivo*

Anna Stelling

The sphingosine-1-phosphate receptor S1PR2 and its downstream signaling pathway is commonly silenced in diffuse large B-cell lymphoma (DLBCL), either by mutational inactivation or through negative regulation by the oncogenic transcription factor FOXP1. In this project, we have examined the upstream regulators of S1PR2 expression and have newly identified the TGF- β /TGF- β R2/SMAD1 axis as critically involved in S1PR2 transcriptional activation. Phosphorylated SMAD1 directly binds to regulatory elements in the S1PR2 locus as assessed by chromatin immunoprecipitation, and the CRISPR-mediated genomic editing of S1PR2, SMAD1 or TGFBR2

in DLBCL cell lines renders cells unresponsive to TGF- β -induced apoptosis. DLBCL clones lacking any one of the three factors have a clear growth advantage *in vitro*, as well as in subcutaneous xenotransplantation models, and in a novel model of orthotopic growth of DLBCL cells in the spleens and bone marrow of MISTRG mice expressing various human cytokines. The loss of S1pr2 induces hyper-proliferation of the germinal center B-cell compartment of immunized mice and accelerates MYC-driven lymphomagenesis in spontaneous and serial transplantation models. The specific loss of Tgfbr2 in murine GC B-cells phenocopies the effects of S1pr2 loss on GC B-cell hyper-proliferation. Finally, we show that SMAD1 expression is aberrantly downregulated in >85% of analyzed DLBCL patients. The combined results uncover an important novel tumor suppressive function of the TGF- β /TGF- β R2/SMAD1/S1PR2 axis in DLBCL (see schematic in Figure 2), and show that DLBCL cells have evolved to inactivate the pathway at the level of SMAD1 expression.



Flori, M., Schmid, C.A., Sumrall, E.T., Tzankov, A., Law, C.W., Robinson, M.D. and Müller, A. The hematopoietic oncoprotein FOXP1 promotes tumor cell survival in diffuse large B-cell lymphoma by repressing S1PR2 signaling. *Blood*, 127:1438-48 (2016).

Stelling, A., Hashwah, H., Bertram, K., Manz, M.G., Tzankov, A. and Müller, A. The tumor suppressive TGF- β /SMAD1/S1PR2 signaling axis is recurrently inactivated in diffuse large B-cell lymphoma. In press, *Blood* (2018).

Figure 2. Schematic summarizing the tumor suppressive properties of the TGF- β /SMAD1/S1PR2 axis in DLBCL. Under physiological conditions, centrocytes and centroblasts express large amounts of S1PR2, which promotes GC confinement due to a gradient of S1P that increases in concentration towards the borders of the GC and leads to apoptosis in GC cells that attempt to exit the GC. In DLBCL, S1PR2 is either mutated (in the GCB subtype) or transcriptionally downregulated by FOXP1 (in the ABC subtype). Loss of S1PR2 thus is an early initiating event in both major subtypes of DLBCL. The expression of S1PR2 is further regulated by TGF- β , which binds to its receptor TGF- β R2 and activates SMAD1 phosphorylation and nuclear translocation. p-SMAD1 binds directly to regulatory elements in the S1PR2 promoter and activates S1PR2 expression; most cases of DLBCL exhibit aberrantly low or absent expression of SMAD1.

The role of myeloid cells in controlling infections with the gastric colonizer and human pathogen *Helicobacter pylori*

Isabelle C. Arnold, Xiaozhou Zhang, Mariella Artola-Boran

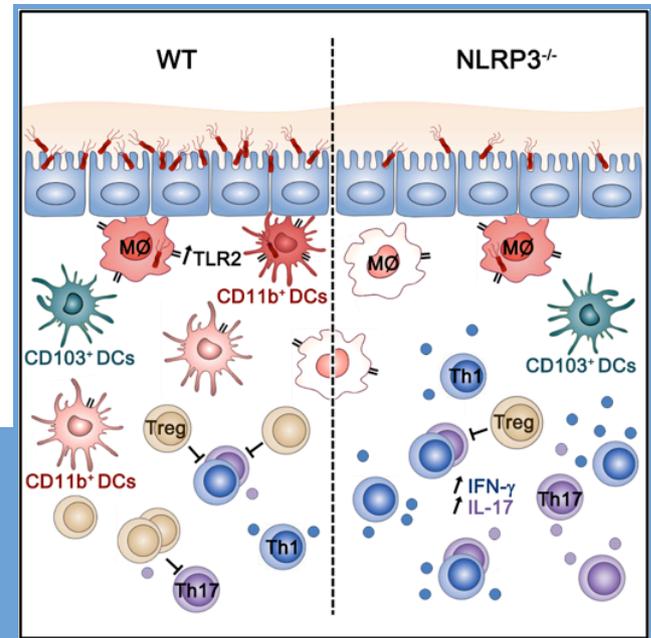
The gastric lamina propria is largely uncharted immunological territory. One project in the lab seeks to unravel the contribution of various populations of myeloid cells (dendritic cells, macrophages, monocytes) to immune control of the gastric pathogen *Helicobacter pylori* and to the immunopathology associated with this infection. We have begun to investigate the evolution and composition of the gastric, small intestinal and colonic lamina propria mononuclear phagocyte system during steady state and infection with *H. pylori*. We show that monocytes, CX3CR1^{hi} macrophages and CD11b⁺ dendritic cells are recruited to the infected stomach in a CCR2-dependent manner (Figure 3). All three populations, but not BATF3-dependent CD103⁺ DCs, sample RFP⁺ *H. pylori*. Mice reconstituted with human hematopoietic stem cells recapitulate several features of the myeloid cell-*H. pylori* interaction. The differentiation in and/or recruitment to gastrointestinal, lung and lymphoid tissues of CD11b⁺ DCs requires NLRP3, but not ASC or caspase-1, during steady state and chronic infection. NLRP3^{-/-} mice fail to generate Treg responses to *H. pylori* and control the infection more effectively than wild type mice. The results demonstrate a non-canonical inflammasome-independent function of NLRP3 in DC development and immune regulation.

Arnold, I.C., Zhang, X., Urban, S., Artola-Boran, M., Manz, M.G., Ottemann, K., and Müller, A. NLRP3 controls the development of gastrointestinal CD11b⁺ dendritic cells during steady state and chronic bacterial infection. *Cell Reports*, 21:3860-3872 (2017).

Figure 3. Schematic showing the consequences of *H. pylori* infection in the NLRP3-proficient and -deficient murine host. Gastric lamina propria CX3CR1^{hi} macrophages and CD11b⁺ DCs sample RFP⁺ *H. pylori* and are displayed in red. NLRP3 is required for the development of CD11b⁺ DCs but not CD103⁺ DCs. NLRP3 is required for Treg development and for the suppression of Th1 responses characterized by high expression of IFN- γ .

In a second project, we have begun to examine the role of eosinophils in *H. pylori* and other gram-negative infections. Eosinophils are predominantly known for their contribution to allergy. In an ongoing study, we are examining the function and regulation of gastrointestinal eosinophils in the steady state and during infection with *Helicobacter pylori* or *Citrobacter rodentium*. We find that eosinophils are recruited to sites of infection, directly encounter live bacteria, and activate a signature transcriptional program; this applies also to human gastrointestinal eosinophils in humanized mice. The genetic or anti-IL-5-mediated depletion of eosinophils results in improved control of the infection, increased inflammation and more pronounced Th1 responses. Eosinophils control Th1 responses via the IFN- γ -dependent upregulation of PD-L1. Furthermore, we find that the conditional loss of IFN- γ R, but not of Myd88 signaling, phenocopies the effects of eosinophil depletion. Eosinophils further possess bactericidal properties that require their degranulation and the deployment of extracellular traps. Our results highlight two novel functions of this elusive cell type and link it to gastrointestinal homeostasis and anti-bacterial defense.

Arnold, I.C., Artola-Boran, M., Tallón de Lara, P., Kyburz, A., Taube, C., Ottemann, K., van den Broek, M., and Müller, A. Eosinophils suppress Th1 responses and restrict bacterially induced gastrointestinal inflammation. In revision, *J Exp Med*.



Trans-maternal exposure to *Helicobacter pylori* induces stable and highly suppressive regulatory T-cells and protects against allergic asthma

Andreas Kyburz, Angela Fallegger

The trans-maternal exposure to tobacco, microbes, nutrients and other environmental factors shapes the fetal immune system through epigenetic processes. The gastric microbe *Helicobacter pylori* represents an ancestral constituent of the human microbiota that causes gastric disorders on the one hand, and is inversely associated with allergies and chronic inflammatory conditions on the other. In this study, we are investigating the consequences of trans-maternal exposure to *H. pylori*, *in utero* and/or during lactation, on the composition of the gastrointestinal microbiota, susceptibility to viral infection, predisposition to allergic asthma, and the development of immune cell populations in the lung and lymphoid organs. We use experimental models of house dust mite-induced allergic asthma and influenza A virus infection along with metagenomics analyses, multi-color flow cytometry and bisulfite pyrosequencing to study the effects of *H. pylori* on allergy severity and immunological and microbiome correlates thereof. We find that perinatal exposure to *H. pylori* extract, or its immunomodulatory molecule VacA, confers robust protective effects against allergic asthma not only in the first (Figure 4), but also the second generation of offspring, but does not increase susceptibility to viral airway infection. Immune correlates of allergy protection include skewing of regulatory over effector T-cells, expansion of Treg subsets expressing CXCR3 or ROR γ t, and demethylation of the FOXP3 locus. The composition and diversity of the gastrointestinal microbiota is measurably affected by perinatal *H. pylori* exposure. We conclude that exposure to *H. pylori* has consequences not only for the carrier, but also for subsequent generations that may be exploited for interventional purposes.

Kyburz, A., Zhang, X., Altobelli, A., Borbet, T., Paul, P., Münz, C., Floess, S., Huehn, J., Cover, T.L., Blaser, M.J., Taube, C. and Müller, A. Trans-maternal exposure to *Helicobacter pylori* induces stable and highly suppressive regulatory T-cells and protects against allergic asthma. In revision for J Allergy and Clinical Immunology.

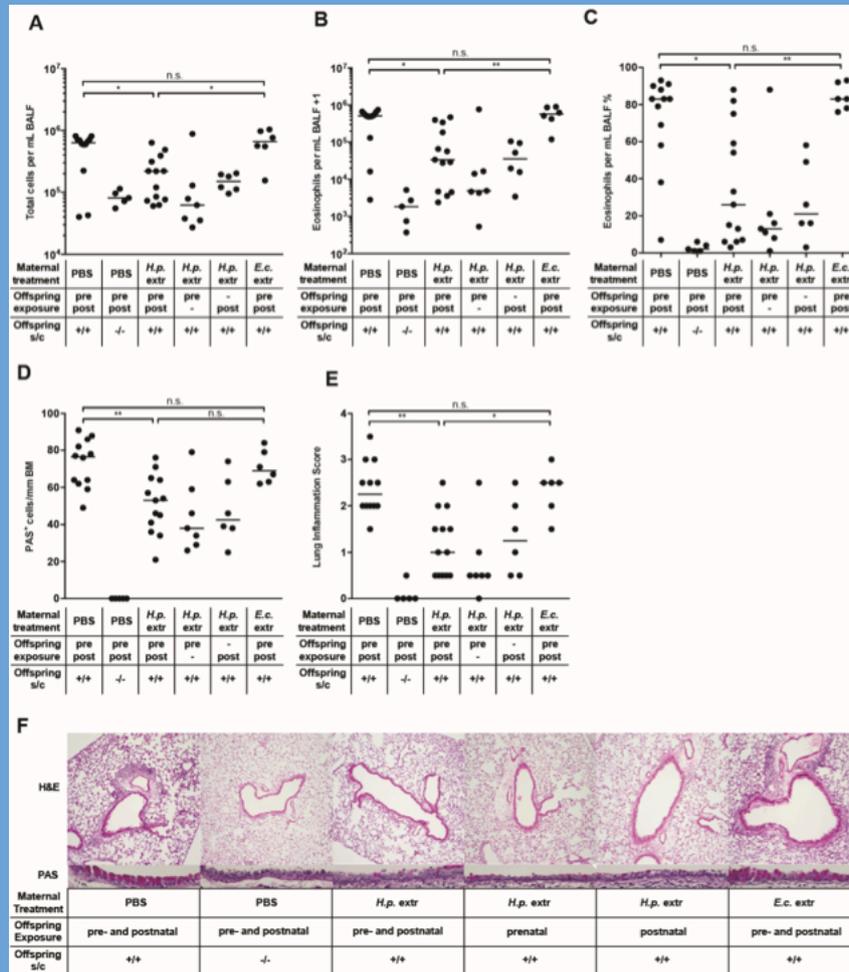


Figure 4. Perinatal trans-maternal exposure to *H. pylori* but not *E. coli* extract protects against house dust mite-induced allergic asthma. Mice were either pre- (pre) and/or postnatally (post/prepost) trans-maternally exposed to *H. pylori*- (*H.p. extr*), *E. coli*-extract (*E.c. extr*) or PBS sham treatment through 2-3 weekly oral gavages of the dams during pregnancy and/or lactation. Litter swaps were conducted at birth wherever necessary to avoid unwanted exposures. At six weeks of age, the offspring was sensitized and challenged (s/c) intranasally with house dust mite (HDM) allergen. Negative controls were sensitized and challenged with PBS only. Bronchoalveolar lavage (BAL) leukocytes and eosinophils were quantified at the study endpoint; lungs were fixed, H&E- or PAS-stained and scored with respect to peribroncheolar and perivascular inflammation and PAS+ goblet cell metaplasia. (A) Total leukocytes in 1 ml of BAL fluid (BALF). (B) Total eosinophils in 1 ml of BALF. (C) Eosinophil frequencies in BALF. (D-F) Pulmonary inflammation and goblet cell metaplasia, as assessed on stained lung sections. BM, basement membrane. Representative sections are shown in F. In A-E, each symbol represents one mouse. The results were pooled from two independent experiments. Horizontal lines indicate medians; an ANOVA with a Dunn's multiple comparisons test was used for calculation of p-values. * $p < 0.05$, ** $p < 0.01$.

The *Helicobacter pylori* immunomodulator VacA suppresses Th17 and favors regulatory T-cell responses by targeting CD11b⁺ dendritic cells and impairing their IL-23 production

Aleksandra Altobelli, Michael Bauer

The gastric bacterium which we study in the lab, *Helicobacter pylori*, has adapted to its human host during at least 100'000 years of co-evolution. Although studied mostly for its inflammatory and even carcinogenic properties in 10% of the infected population, *H. pylori* also has beneficial effects on its human host. These include strong protective effects against allergic diseases such as allergic asthma, rhinitis and atopic dermatitis, and chronic inflammatory diseases such as inflammatory bowel disease. The beneficial effects are most evident in children and young adults, and can be mimicked by experimental infection of mice in early life, i.e. at 7 days of age. We have lately extensively used this neonatal infection model to study the role of the *H. pylori* immunomodulator VacA in immunity and immune tolerance to *H. pylori*. We find that VacA targets various gastric populations of antigen-presenting cells, including CD11b⁺ DCs, which are recruited in large numbers to the gastric mucosa and locally sample *H. pylori*. Whereas CD11b⁺ DCs exposed to VacA-deficient bacteria produce large amounts of IL-23, this is not the case with wild type (VacA-proficient) *H. pylori*, indicating that VacA suppresses IL-23 expression in these cells. Consequently, gastric Th17 responses are reduced in WT-infected mice, as determined by intracellular cytokine and Ror γ t staining, and VacA-deficient bacteria are cleared more readily than WT *H. pylori*. The suppression of gastric Th17 responses is accompanied by higher frequencies of peripherally induced regulatory T-cells (iTregs), in particular those subsets expressing Ror γ t, in the gastric mucosa as well as systemically in the spleen. The preferential VacA-dependent generation of Ror γ t-expressing T-regulatory over Th17 responses provides a mechanistic explanation for the observation that VacA proficiency is required for the beneficial effects of *H. pylori* infection in settings of allergic asthma and other chronic allergic and inflammatory conditions.

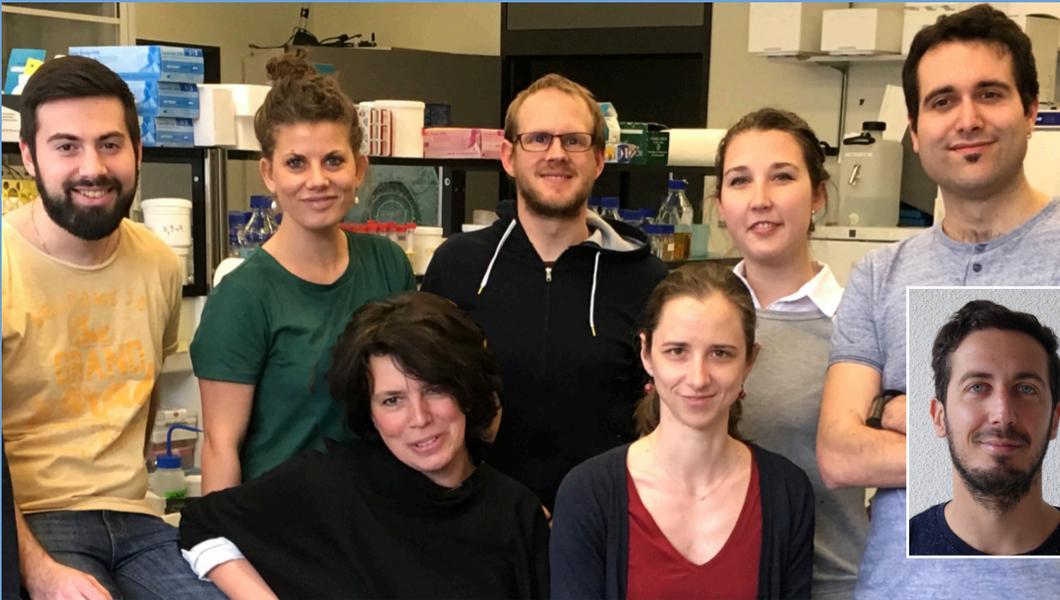
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LORENZA PENENGO



UBIQUITIN CONTROL OF GENOME STABILITY



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*left 2017

Our research focuses on the role of ubiquitination and ubiquitin-like modifications in the regulation of genome stability. We aim to understand how cells react to harmful conditions – genotoxic stress, inflammation, viral infection – by defining the ubiquitin and ubiquitin-like signature that marks chromatin in different cellular contexts, and by understanding how these ubiquitin-based signals are further decoded into functional outputs.

Ubiquitin conjugation is a widely-used protein modification that regulates virtually all cellular pathways, including those relevant for cancer cell proliferation and genome integrity such as the DNA damage response (DDR) and DNA replication. Ubiquitin is covalently conjugated to lysine (K) residues on target protein by a multistep process, leading to the formation of different polyubiquitin chains that result in a multitude of functional outcomes, including both proteolytic and non-proteolytic activities. A paradigmatic example of the non-proteolytic role of ubiquitin is represented by the DDR signalling pathway activated by DNA double strand breaks (Figure 1), which is initiated by the apical kinase ATM (ataxia-telangiectasia mutated). ATM-dependent phosphorylation events are rapidly followed by chromatin ubiquitination on histone H2As, mediated by the E3 ligases RNF8 and RNF168. Ubiquitinated H2As represent the docking site for downstream factors, such as 53BP1 and the BRCA1 complex, which are required for promoting DNA repair. The key role of RNF168 has been further demonstrated by the identification of *RNF168* gene mutations leading to a disorder called RIDDLE syndrome which is characterized by radiosensitivity, immunodeficiency and cancer predisposition, clearly identifying this ubiquitin-mediated pathway as a potential therapeutic target. Indeed, the ubiquitin system is particularly attractive as new anticancer target, thanks to the presence of specific enzymatic – and therefore “drugable” – activities modulating different cellular pathways. Inhibiting key ubiquitin-related DNA repair factors could potentiate commonly used anticancer drugs that induce genotoxic stress. However, reaching this goal requires mechanistic understanding of ubiquitin-mediated control of genome stability, beyond the few molecular events known to date.

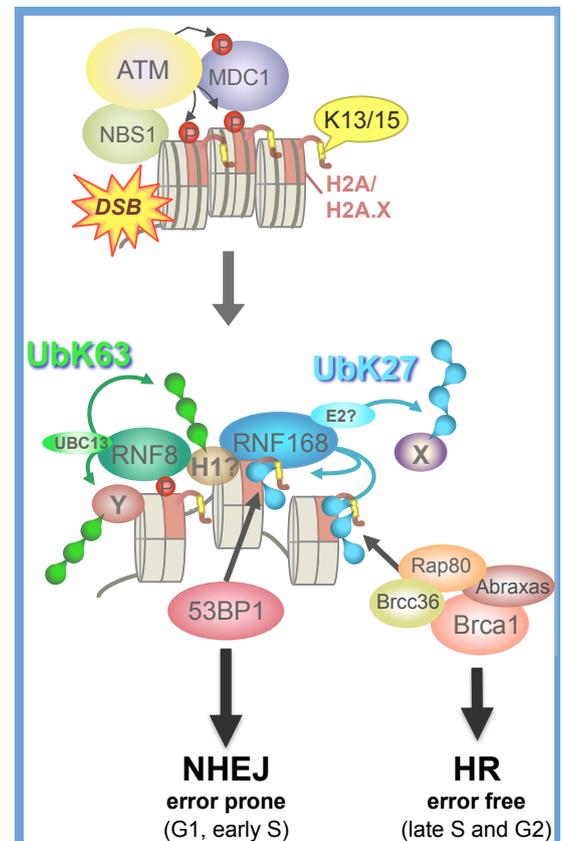


Figure 1. Formation of DSBs by genotoxic treatments induces phosphorylation events targeting different substrates – including histones H2A and H2A.X – thereby generating the docking sites for downstream DDR factors. These events allow the recruitment of ubiquitinating enzymes, namely RNF8 and RNF168, which results in extensive ubiquitination of the surrounding chromatin. Major targets of RNF168-induced ubiquitination are histones of the H2A family, which are modified on the K13/K15 site of the N-terminal tail of the protein. This ubiquitination event is strictly dependent on a non-canonical type of ubiquitination, linked through the K27 residue of Ub, and is required for the recruitment to DDR foci of factors – i.e. 53BP1 and BRCA1 - responsible for activating downstream DNA repair process via either non-homologous end-joining (NHEJ) or homologous recombination (HR).

RNF168 triggers non-canonical ubiquitination of chromatin to signal DNA damage

Marco Gatti

For a long time, it has been assumed that when cells are exposed to genotoxic agents, chromatin histones H2A and H2A.X are modified by a specific type of modification, i.e. K63-linked ubiquitination on the C-terminal K119 of H2As, by the ubiquitin ligase RNF168. This

poses the issue of specificity in the system, due to the abundance of H2A ubiquitination (10-15% of H2As are ubiquitinated on K119). While interrogating this issue, we discovered that RNF168 targets non-canonical sites on histone H2A, namely K13 and K15, generating novel chromatin marks at the N-terminal tail of the protein (H2AK13ub and H2AK15ub, Figure 2A). Moreover, we showed that an additional level of specificity is given by the peculiar type of ubiquitination required to trigger RNF168 activity, which is mediated by K27 linkage of ubiquitin (Figure 2B). All together these findings account for the specificity of the signal triggered by DNA double strand breaks and involving the ubiquitin ligase RNF168.

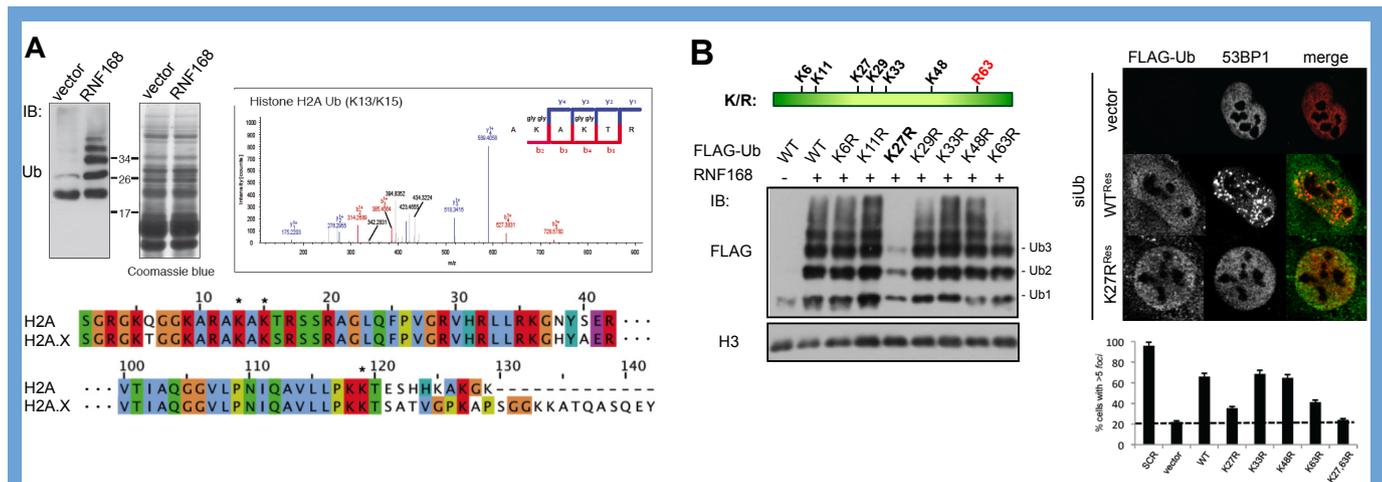


Figure 2.

A. Mass spectrometric analysis of chromatin extracts derived from cells expressing RNF168 revealed a novel ubiquitination site on histone H2As, formed by K13/K15 residues, which is embedded in a highly-conserved sequence.

B. Schematic example of the Ub K-R mutants used in the experiment. Chromatin ubiquitination is considerably impaired in cells expressing the K27R mutant of Ub (left panel). Transient depletion of Ub (siRNA Ub) completely blocks the recruitment of 53BP1 to the sites of lesions (see vector). DDR foci are restored by the expression of a siRNA-resistant form of Ub (WT^{Res}), but not by the K27R mutant (K27R^{Res}), clearly indicating a pivotal role of the K27 linkage in this process.

Role of post-translational modifications in DNA damage-induced chromatin remodelling

Franziska Pfister

Maintaining genome stability is crucial for all living cells and organisms. The repair of the highly cytotoxic DNA double-strand breaks is based on a variety of factors that modify chromatin structure, such as kinases and ubiquitin ligases. Our previous studies demonstrated that H2AK13Ub/H2AK15Ub promoted by RNF168 is a key event in this pathway. However, how the RNF168 pathway is functionally implicated in determining the repair pathway choice critical to genome maintenance by promoting either the error-prone NHEJ – *via* 53BP1 – or the error-free HR – *via* BRCA1 – is still missing (Figure 3). By using different experimental approaches, we discovered and characterized novel unprecedented modifications of chromatin, which contribute to explain the fine modulation of DNA repair and the pathway choice in different phases of the cell cycle.

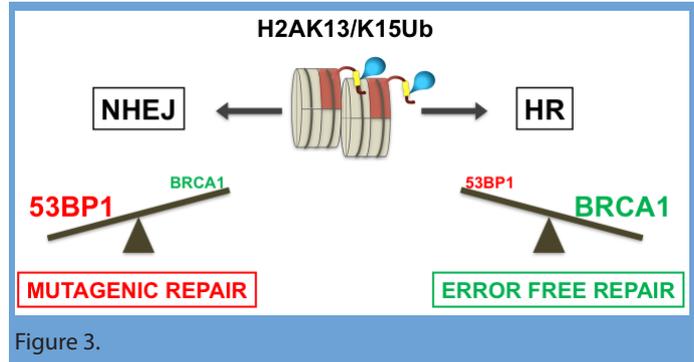


Figure 3.

Interferon Stimulated Gene 15 – ISG15 – as novel factor involved in genome stability

Maria Chiara Raso

Although the relevance of ubiquitin has been already defined in different processes linked to genome stability, such as DNA replication and DNA damage response, the role of other ubiquitin-like modifiers is still elusive. One of these modifiers is the *Interferon Stimulated Gene 15*, ISG15, which is strongly induced during pathogen infection via interferon type-1, being part of the innate immunity (Figure 4). Interestingly, ISG15 and its conjugation - a process called

ISGylation - are also induced by different genotoxic treatments, such as camptotecin (CPT), hydroxyurea (HU) and UV light. Recently reported to be deregulated in most human malignancies, ISG15 is emerging as an important oncoprotein and a potential diagnostic and therapeutic target for cancer. Even though many proteomic studies identified different proteins involved in DNA repair and DNA replication as potential targets of ISG15, a functional and comprehensive analysis of these factors is still missing. We aimed at further expanding these studies, by investigating the role of ISG15 – and its targets – in the maintenance of genome integrity, by focusing on their specific role in the DNA damage response and DNA replication.

ISG15

- Its expression is highly promoted by Interferon type-1
- Is involved in innate immunity
- Is activated by viruses and bacterial infection, it targets and inactivates pathogen proteins
- Is induced by genotoxic stress
- Is over-expressed in many types of tumors
- Targets different factors involved in genome stability and DNA repair

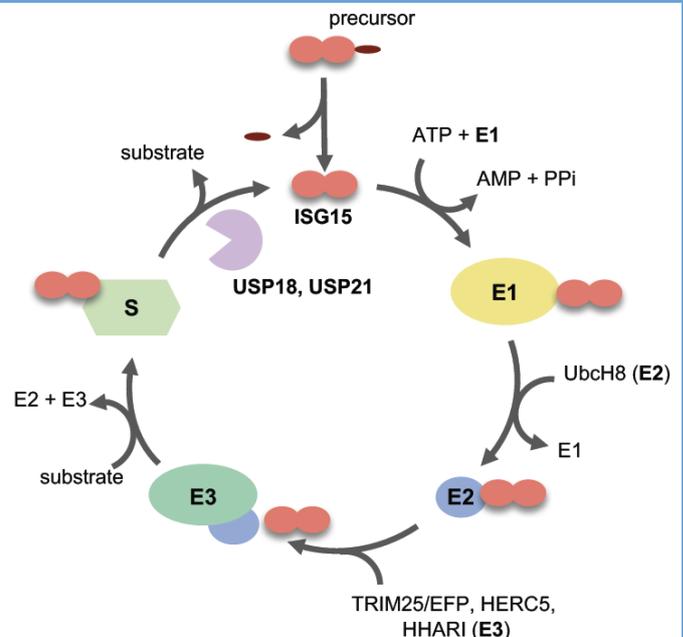


Figure 4. Schematic representation of the three-step cascade of the ISGylation process, involving E1 activating enzyme, E2 conjugating enzyme and E3 ligase.

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ALESSANDRO A. SARTORI



DNA DAMAGE AND REPAIR: FROM MOLECULAR MECHANISMS TO TARGETED THERAPY



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Studying the causes and consequences of DNA damage on the molecular and cellular level is a major topic of research within cancer biology. DNA damage not only causes cancer but is also used as a means to cure cancers through radio- or chemotherapy and is also responsible for the side effects of these treatments. Therefore, a better understanding of the underlying mechanisms activated in response to DNA damage is of utmost importance for the development of novel anti-cancer therapies.

The main focus of our laboratory is to gain molecular and genetic insights into the interplay between DNA damage signaling and repair in human cells. Within this broad area of research, we are particularly interested in the repair of DNA double-strand breaks (DSBs), the most hazardous lesions a cell can encounter. A detailed knowledge of the players acting in DSB repair processes is crucial if we are to improve current cancer therapies and suggest novel strategies to fight this disease.

DSBs are repaired by two mechanisms: homologous recombination (HR) and non-homologous end-joining (NHEJ). The human CtIP pro-

tein plays a critical role in DSB repair pathway choice by promoting DNA-end resection and HR (Figure 1). We are employing several approaches to further our understanding on the regulation of CtIP and other key DSB repair proteins by multiple mechanisms, involving cell cycle control, post-translational modifications (PTMs) and protein-protein interactions (PPIs). Besides the exciting prospect of identifying novel factors involved in DSB repair by high-throughput proteomic screens, we use a combination of biochemistry, cell biology and genetics to address the function of CtIP and other factors implicated in the maintenance of genome stability (Figure 2).

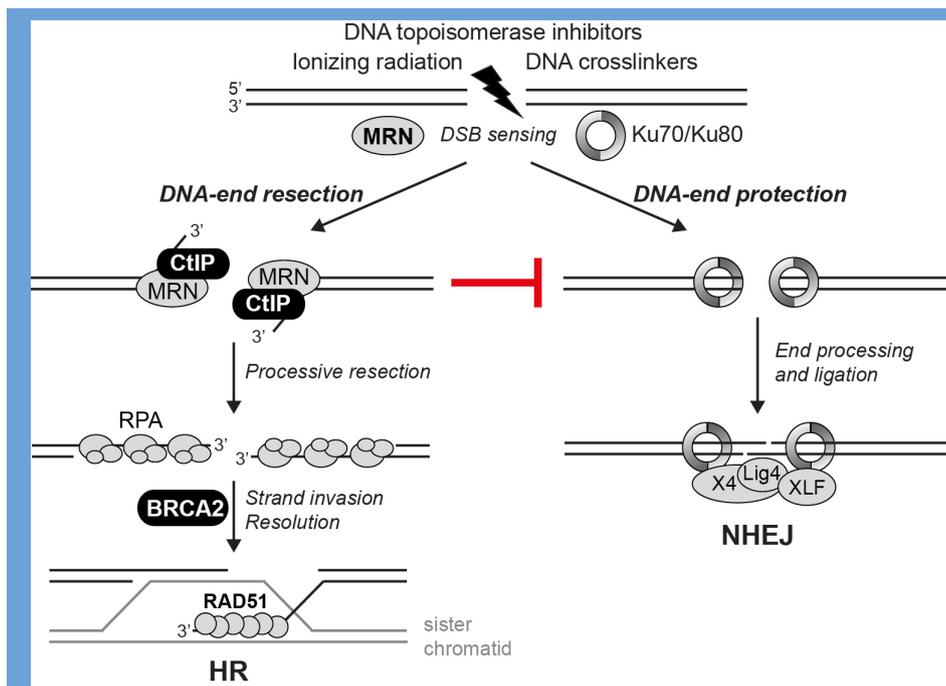


Figure 1. Mechanistic model depicting the role of CtIP in DSB repair pathway choice. DSBs induced by a variety of exogenous agents used in cancer therapy are sensed by either the MRN complex or the Ku heterodimer. CtIP and MRN are essential for initiating DNA-end resection, the first step in HR, and suppressing NHEJ. In HR, initial processing of DSBs is followed by processive resection through the combined action of DNA helicases and nucleases. The 3'-single-stranded DNA (ssDNA) is protected from degradation through the binding of RPA. BRCA2 promotes replacement of RPA by the RAD51 recombinase, which is required for strand invasion of the sister chromatid promoting D-loop formation and further downstream steps in HR.

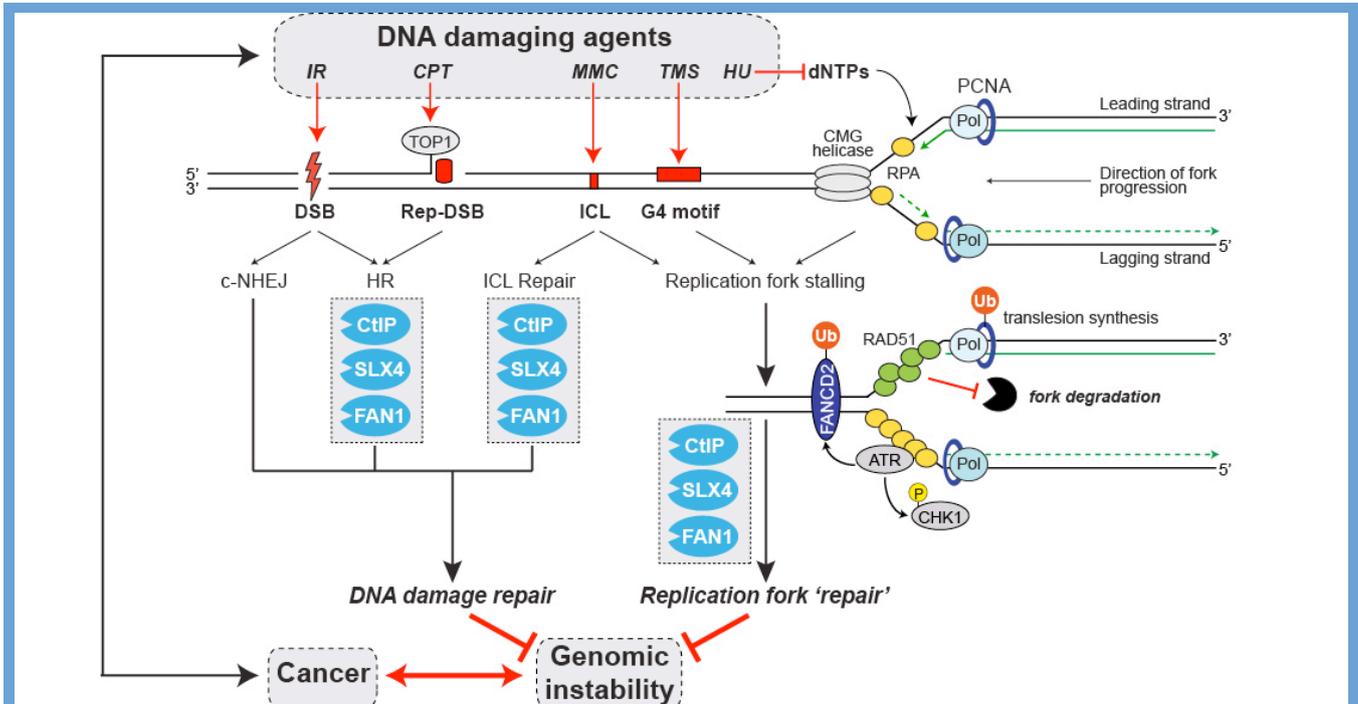


Figure 2. CtlP, FAN1 and SLX4 DNA repair nucleases: Caretakers required for the maintenance of genome integrity and cancer avoidance. Schematic illustration highlighting the roles of CtlP, SLX4 and FAN1 proteins in response to a variety of DNA damaging agents causing different types of DNA lesions. Abbreviations: IR, ionizing radiation; CPT, camptothecin; MMC, mitomycin C; TMS, telomestatin; HU, hydroxyurea; DSB, double-strand break; Rep-DSB, replication-dependent DSB; ICL, DNA interstrand crosslink; G4, guanine-rich DNA sequence motifs potentially forming four-stranded G-quadruplex structures during DNA replication; CMG, Cdc45/Mcm2-7/GINS; Pol, DNA polymerase; c-NHEJ, classical non-homologous end-joining; HR, homologous recombination; Ub, ubiquitin; P, phospho.

The regulation of CtIP by post-translational modifications

Sarah-Felicitas Himmels

Several recent studies have indicated that CtIP is under tight regulation by a number of post-translational modifications, including phosphorylation and ubiquitylation. However, while it was established that phosphorylation of CtIP is required for the regulation of DNA-end resection during the cell cycle, it is still largely unknown how ubiquitylation and deubiquitylation controls CtIP function. Our most recent findings point toward a crucial role for the cullin-3 (CUL3) E3 ubiquitin ligase in the regulation of CtIP protein stability. In brief, we discovered that the CUL3 substrate adaptor Kelch-

like protein 15 (KLHL15) K48 ubiquitinates CtIP to promote its proteasomal degradation. Accordingly, we observed that KLHL15 is a critical regulator of CtIP-mediated DNA-end resection and governs the balance between HR and NHEJ. Given that protein ubiquitination is a reversible process, we aim to investigate, whether there is a deubiquitinase (DUB) countering CtIP proteasomal degradation. A high-throughput flow cytometry-based screening approach employing a targeted siRNA library revealed FAM63A and FAM63B as potential regulators of CtIP (Figure 3). Interestingly, FAM63A and FAM63B belong to a newly identified DUB family, called MINDY, which is characterized by its strong preference to cleave long K48 ubiquitin chains, yet its physiological function remain completely unknown.

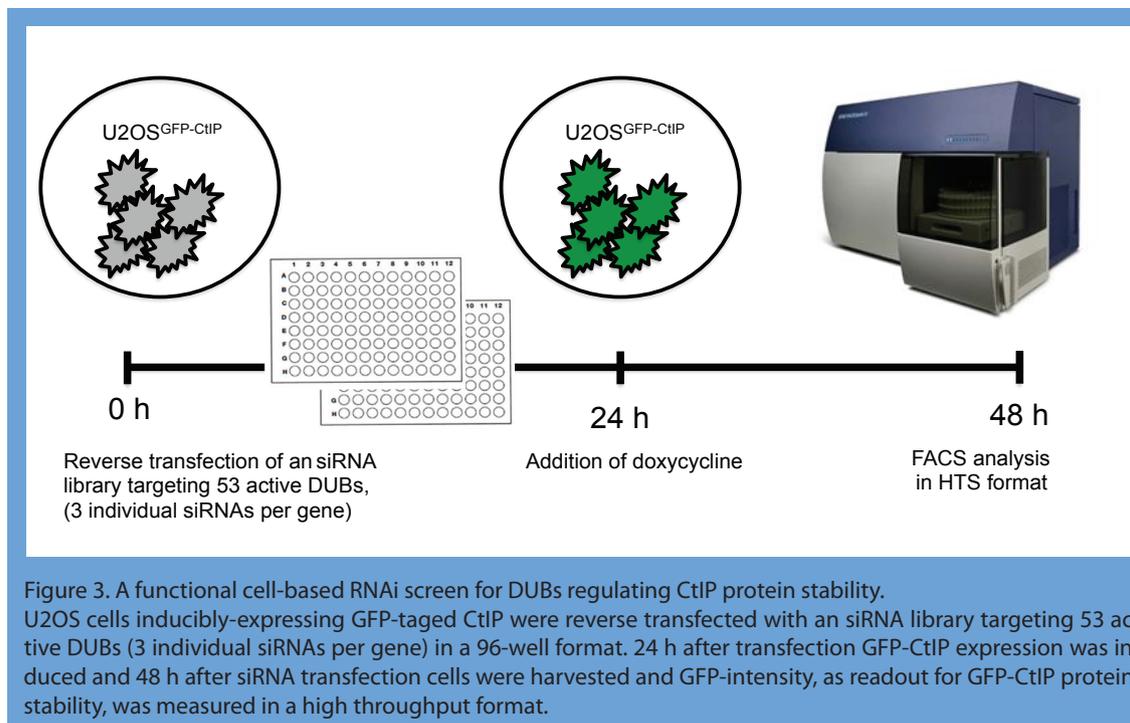


Figure 3. A functional cell-based RNAi screen for DUBs regulating CtIP protein stability. U2OS cells inducibly-expressing GFP-tagged CtIP were reverse transfected with an siRNA library targeting 53 active DUBs (3 individual siRNAs per gene) in a 96-well format. 24 h after transfection GFP-CtIP expression was induced and 48 h after siRNA transfection cells were harvested and GFP-intensity, as readout for GFP-CtIP protein stability, was measured in a high throughput format.

The role of the PIN1 isomerase in the maintenance of genome stability

Julia Godau

An accurate regulation of the DNA damage response (DDR) by phosphorylation-dependent signaling is absolutely pivotal to trigger correct repair of the DNA. Inappropriate regulation can have detrimental effects for the cell fate, hence incorrect repair can cause tumorigenesis. However, surprisingly little is known about the DDR phosphorylation-network and its fine-tuning. The unique enzyme called PIN1 (Peptidyl-prolyl cis-trans isomerase NIMA-interacting 1) acts on the proteins, which has been previously phosphorylated at specific sites, resulting in conformational changes. A change between cis or trans conformation of amino acid residues in protein can have enormous consequences for its target function or stability.

This post-phosphorylation action of PIN1 depicts a novel signaling mechanism. Although the assumption is obvious to suggest that PIN1 is involved in the DNA damage response (DDR) the implication of PIN1 in this process is not understood. Recently, our lab identified a new role for PIN1 to control double-strand break repair pathway choice through promoting CtIP isomerization (Figure 4). Intriguingly, using a proteomic screen, we discovered several key DDR factors as potential substrates of PIN1, including SLX4 (Figure 4). SLX4 is a multi-domain scaffold protein for several structure-specific nucleases involved in the cleavage of interstrand-crosslinks and resolution of Holliday junctions as well as in the trimming of telomeric loops. Our data suggests that PIN1 indeed binds to SLX4 in a phosphorylation-dependent manner. In summary, we plan to investigate the role of site-specific SLX4 phosphorylation and isomerization in the DDR, further highlighting the importance of PIN1 in the maintenance of genome stability.

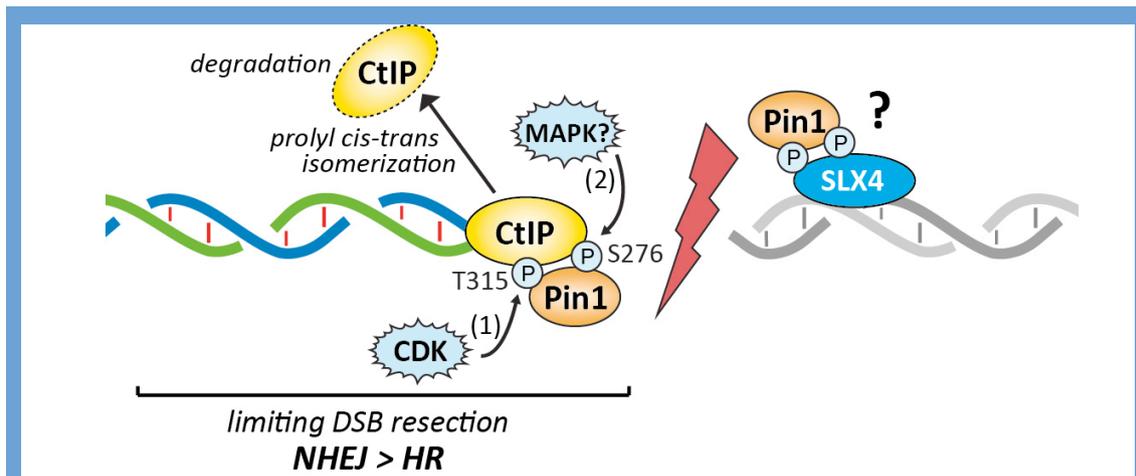


Figure 4. Model of PIN1-mediated restriction of DNA-end resection.

During S/G2 phase of the cell cycle, CtIP is phosphorylated at residue T315 by CDK2 and/or CDK1. This priming phosphorylation event is necessary for PIN1 binding to CtIP. Upon DSB formation, CtIP is further phosphorylated at residue S276 by an as-yet-unknown proline-directed kinase (e.g., MAPKs) and subsequently isomerized by PIN1. This conformational change promotes CtIP polyubiquitination, leading to its proteasomal degradation. In this way, PIN1 counteracts DNA-end resection and hence HR, thereby facilitating NHEJ. Our proteomics screen revealed SLX4 as an additional PIN1 substrate. We are currently exploiting whether PIN1-mediated SLX4 isomerization controls a specific aspect of SLX4 function in genome stability. Image modified from "Prolyl isomerization: a new PIN code for DSB repair" by Sartori and Steger (Cell Cycle. 2013;12(17):2717-2718).

Characterizing the function of human CtIP at stalled replication forks

Sara Przetocka

Various cellular mechanisms cooperate to ensure faithful DNA replication and maintain genome stability. However, replication forks are frequently challenged by exogenous or endogenous DNA damage. This leads to transient slowing or stalling of replication forks, which is defined as replication stress. Homologous Recombination (HR) and the Fanconi Anemia (FA) pathways act in collaboration in order to secure fork stabilization and promote fork recovery. Human CtIP is most widely recognized for its essential function in DNA-end resection and homology-directed repair of DNA double-strand breaks. Furthermore, increasing evidence implicates CtIP as a critical factor for the maintenance of genome stability owing to its roles in transcriptional regulation, the DNA damage response and cell cycle checkpoint control. Recently, CtIP was reported to promote the recovery of stalled replication forks suggesting that CtIP also participates in the response to replication stress. However, our understanding of the role of CtIP in facilitating accurate DNA replication and preventing replication stress-induced genomic instability is still very limited. Using DNA fiber spreading, we are currently analyzing the functional relationship between CtIP and HR and FA proteins response to replication stress.

FAN1: Molecular insights into a multifunctional DNA repair nuclease

Antonio Porro

FANCD2-associated nuclease 1 (FAN1) was originally identified in a genome-wide screen for proteins interacting with the mismatch repair factor MLH1. In response to DNA interstrand crosslink (ICL)-inducing drugs, FAN1-deficient cells display increased frequency of chromosomal aberrations and hypersensitivity. FAN1 was proposed to act as a 5'-flap endonuclease during ICL unhooking. At sites of ICL lesions but also at stalled replication forks, FAN1 has been reported to interact via its ubiquitin-binding zinc finger (UBZ) domain with monoubiquitylated FANCD2, a key factor of the Fanconi anemia pathway. We have recently revised this concept and demonstrated that FAN1 recruitment of to arrested forks is mediated by ubiquitylated PCNA rather than by ubiquitylated FANCD2. Therefore, our study unveils a role for FAN1 outside of the FA pathway. The FAN1-PCNA interaction requires the UBZ domain of FAN1 but also a newly identified non-canonical PCNA-interacting protein (PIP) box motif (Figure 5). Thus, FAN1 can be considered as a genuine reader of ubiquitylated PCNA that may help to elicit specific functions of PCNA at damaged replication forks. In addition, we found that FAN1 antagonizes replication stress-induced genomic instability occurring in *BRCA*-deficient cells, raising the exciting prospect of targeting FAN1 as a novel therapeutic strategy in patients with *BRCA*-mutated breast and ovarian cancer.

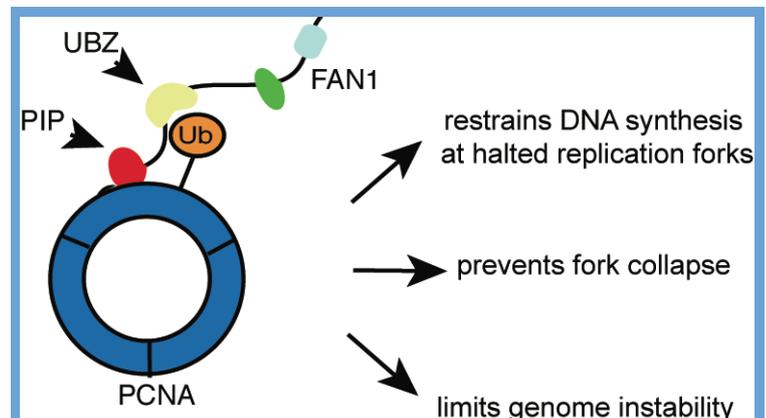


Figure 5. Scheme of the FAN1:ub-PCNA interaction and its biological implications (from Porro et al., Nat Commun. 2017;8:1073)

Towards the design of synthetic cell-penetrating peptides targeting DNA double-strand break repair

Anika Trenner

Efficient DNA repair mechanisms are pivotal to counteract genomic instability, a hallmark of almost all cancer cells. DNA double-strand breaks (DSBs) are the most hazardous lesions a cell can encounter as a single unrepaired DSB leads to cell death, whereas erroneous repair results in mutations, which in turn can promote carcinogenesis. On the other hand, conventional cancer treatment by radiotherapy and certain chemotherapeutic drugs takes advantage of the cytotoxic properties of DSBs. However, these agents often lack selectivity for tumour cells resulting in severe side effects for the patients, thus compromising their therapeutic potential. Hence, new strategies are key to the development of novel compounds that display synergistic effects with standard anti-cancer drugs by spe-

cifically targeting DSB repair (Figure 6). In particular, homologous recombination (HR) as a repair mechanism for DSBs is indispensable for cancer cell survival. Importantly, key HR factors, including CtIP and BRCA2, are primarily governed by a number of protein-protein interactions (PPIs). Consequently, specific targeting of these PPI interfaces could significantly improve the efficacy of conventional anti-cancer therapies. However, inhibition of PPIs has proven challenging as PPI interfaces commonly do not support binding of small drug-like molecules. In contrast, peptide-based inhibitors of PPIs are considered promising but are frequently confined by conformational and proteolytical instability as well as cell membrane impermeability. Importantly, artificial backbone modifications and the use of cell-penetrating peptides (CPPs) as delivery vectors were shown to significantly improve pharmaceutical properties of peptides. We aim to design peptide-based inhibitors mimicking essential CtIP and BRCA2 PPI interfaces, whose potencies are improved by the use of the CPP nona-arginine (R9) and various backbone-modifying stapling techniques.

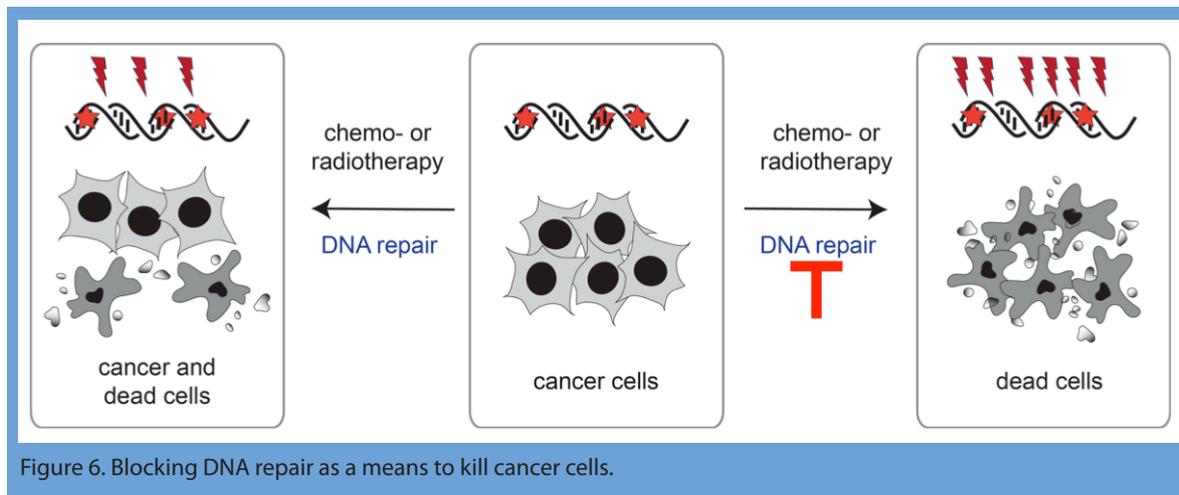


Figure 6. Blocking DNA repair as a means to kill cancer cells.

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ADMINISTRATIVE, IT AND LABORATORY SUPPORT

This section of the Institute fulfils multiple roles.

The administrators deal with personnel and financial matters, and co-ordinate the Cancer Network Zurich, and the PhD Program in Cancer Biology.

The IT section updates and maintains the Institute's computer facilities and is responsible for the creation and updating of the websites of the Institute and the above-named centres.

The laboratory support section looks after the day-to-day running of the Institute.

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PUBLICATIONS

2016

Acosta-Ramirez, Elizabeth; Tram, Cynthia; Kampen, Rachel M; Tillman, Melanie R; Schwendener, Reto A; Xing, Zhou; Halperin, Scott A; Wang, Jun (2016). Respiratory macrophages regulate CD4 T memory responses to mucosal immunization with recombinant adenovirus-based vaccines. *Cellular Immunology*, 310:53-62.

Adam, Ronja; Spier, Isabel; Zhao, Bixiao; Kloth, Michael; Marquez, Jonathan; Hinrichsen, Inga; Kirfel, Jutta; Tafazzoli, Aylar; Horpaopan, Sukanya; Uhlhaas, Siegfried; Stienen, Dietlinde; Friedrichs, Nicolaus; Altmüller, Janine; Laner, Andreas; Holzapfel, Stefanie; Peters, Sophia; Kayser, Katrin; Thiele, Holger; Holinski-Feder, Elke; Marra, Giancarlo; Kristiansen, Glen; Nöthen, Markus M; Büttner, Reinhard; Möslin, Gabriela; Betz, Regina C; Brieger, Angela; Lifton, Richard P; Aretz, Stefan (2016). Exome sequencing identifies biallelic MSH3 germline mutations as a recessive subtype of colorectal adenomatous polyposis. *American Journal of Human Genetics*, 99(2):337-351.

Ahuja, Akshay K; Jodkowska, Karolina; Teloni, Federico; Bizard, Anna H; Zellweger, Ralph; Herrador, Raquel; Ortega, Sagrario; Hickson, Ian D; Altmeyer, Matthias; Mendez, Juan; Lopes, Massimo (2016). A short G1 phase imposes constitutive replication stress and fork remodelling in mouse embryonic stem cells. *Nature Communications*, 7:10660-10671.

Anand, Roopesh; Ranjha, Lepakshi; Cannavo, Elda; Cejka, Petr (2016). Phosphorylated CtIP functions as a co-factor of the MRE11-RAD50-NBS1 endonuclease in DNA end resection. *Molecular Cell*, 64(1):940-950.

Arnold, Isabelle C; Müller, Anne (2016). *Helicobacter pylori*: does gastritis prevent colitis? *Inflammatory Intestinal Diseases*, 1(3):102-112.

Bregenhorn, Stephanie; Kallenberger, Lia; Artola-Borán, Mariela; Peña-Díaz, Javier; Jiricny, Josef (2016). Non-canonical uracil processing in DNA gives rise to double-strand breaks and deletions: relevance to class switch recombination. *Nucleic Acids Research*, 44(6):2691-2705.

Budhathoki, Jagat B; Maleki, Parastoo; Roy, William A; Janscak, Pavel; Yodh, Jaya G; Balci, Hamza (2016). A comparative study of g-quadruplex unfolding and DNA reeling activities of human RECQ5 helicase. *Biophysical Journal*, 110(12):2585-2596.

Erzinger, Melanie M; Bovet, Cédric; Hecht, Katrin M; Senger, Sabine; Winiker, Pascale; Sobotzki, Nadine; Cristea, Simona; Beerenwinkel, Niko; Shay, Jerry W; Marra, Giancarlo; Wollscheid, Bernd; Sturla, Shana J (2016). Sulforaphane preconditioning sensitizes human colon cancer cells towards the bioreductive anticancer prodrug PR-104A. *PLoS ONE*, 11(3):e0150219.

Ferrari, Stefano; Gentili, Christian (2016). Maintaining genome stability in defiance of mitotic DNA damage. *Frontiers in Genetics*, 7:128.

Ferretti, Lorenza P; Himmels, Sarah-Felicitas; Trenner, Anika; Walker, Christina; von Aesch, Christine; Eggenschwiler, Aline; Murina, Olga; Enchev, Radoslav I; Peter, Matthias; Freire, Raimundo; Porro, Antonio; Sartori, Alessandro A (2016). Cullin3-KLHL15 ubiquitin ligase mediates CtIP protein turnover to fine-tune DNA-end resection. *Nature Communications*, 7:12628.

Flori, Michael; Schmid, Corina A; Sumrall, Eric T; Tzankov, Alexandar; Law, Charity W; Robinson, Mark D; Müller, Anne (2016). The hematopoietic oncoprotein FOXP1 promotes tumor cell survival in diffuse large B-cell lymphoma by repressing S1PR2 signaling. *Blood*, 127(11):1438-1448.

Galanos, Panagiotis; Vougas, Konstantinos; Walter, David; Polyzos, Alexander; Maya-Mendoza, Apolinar; Haagensen, Emma J; Kokkalis, Antonis; Roumelioti, Fani-Marlen; Gagos, Sarantis; Tzetzis, Maria; Canovas, Begoña; Igea, Ana; Ahuja, Akshay K; Zellweger, Ralph; Havaki, Sofia; Kanavakis, Emanuel; Kletsas, Dimitris; Roninson, Igor B; Garbis, Spiros D; Lopes, Massimo; Nebreda, Angel; Thanos, Dimitris; Blow, J Julian; Townsend, Paul; Sorensen, Claus Storgaard; Bartek, Jiri; Gorgoulis, Vassilis G (2016). Chronic p53-independent p21 expression causes genomic instability by deregulating replication licensing. *Nature Cell Biology*, 18(7):777-789.

Hermans, Nicolaas; Laffeber, Charlie; Cristovão, Michele; Artola-Borán, Mariela; Mardenborough, Yannicka; Ikpa, Pauline; Jaddoe, Aruna; Winterwerp, Herrie H K; Wyman, Claire; Jiricny, Josef; Kanaar, Roland; Friedhoff, Peter; Lebbink, Joyce H G (2016). Dual daughter strand incision is processive and increases the efficiency of DNA mismatch repair. *Nucleic Acids Research*, 44(14):6770-6786.

Hess, Jeannine; Patra, Malay; Jabbar, Abdul; Pierroz, Vanessa; Konatschnig, Sandro; Spingler, Bernhard; Ferrari, Stefano; Gasser, Robin B; Gasser, Gilles (2016). Assessment of the nematocidal activity of metallocenyl analogues of monepantel. *Dalton Transactions*, 45(44):17662-17671.

Hess, Jeannine; Patra, Malay; Pierroz, Vanessa; Spingler, Bernhard; Jabbar, Abdul; Ferrari, Stefano; Gasser, Robin B; Gasser, Gilles (2016). Synthesis, Characterization, and Biological Activity of Ferrocenyl Analogues of the Anthelmintic Drug Monepantel. *Organometallics*, 35(19):3369-3377.

Himmels, Sarah-Felicitas; Sartori, Alessandro A (2016). Controlling DNA-end resection: an emerging task for ubiquitin and SUMO. *Frontiers in Genetics*, 7:152.

Imstepf, Sebastian; Pierroz, Vanessa; Raposinho, Paula; Felber, Michael; Fox, Thomas; Fernandes, Céilia; Gasser, Gilles; Santos, Isabel R; Alberto, Roger (2016). Towards ^{99m}Tc-based imaging agents with effective doxorubicin mimetics: a molecular and cellular study. *Dalton Transactions*, 45(33):13025-13033.

Kemmerich, Felix E; Daldrop, Peter; Pinto, Cosimo; Levikova, Maryna; Cejka, Petr; Seidel, Ralf (2016). Force regulated dynamics of RPA on a DNA fork. *Nucleic Acids Research*, 44(12):5837-5848.

Kyburz, Andreas; Müller, Anne (2016). The gastrointestinal tract microbiota and allergic diseases. *Digestive Diseases*, 34(3):230-243.

Lafranchi, Lorenzo; Sartori, Alessandro A (2016). The ubiquitin ligase APC/C(Cdh1) puts the brakes on DNA-end resection. *Molecular & Cellular Oncology*, 2(4):e1000696.

Lu, Huiming; Shamanna, Raghavendra A; Keijzers, Guido; Anand, Roopesh; Rasmussen, Lene Juel; Cejka, Petr; Croteau, Deborah L; Bohr, Vilhelm A (2016). RECQL4 promotes DNA end resection in repair of DNA double-strand breaks. *Cell Reports*, 16(1):161-173.

Maglietta, Antonella; Maglietta, Rosalia; Staiano, Teresa; Bertoni, Ramona; Ancona, Nicola; Marra, Giancarlo; Resta, Leonardo (2016). The immune landscapes of polypoid and nonpolypoid precancerous colorectal lesions. *PLoS ONE*, 11(7):e0159373.

Oh, Julyun; Al-Zain, Amr; Cannavo, Elda; Cejka, Petr; Symington, Lorraine S (2016). Xrs2 dependent and independent functions of the Mre11-Rad50 complex. *Molecular Cell*, 64(2):405-415.

Ölmezer, Gizem; Levikova, Maryna; Klein, Dominique; Falquet, Benoît; Fontana, Gabriele Alessandro; Cejka, Petr; Rass, Ulrich (2016). Replication intermediates that escape Dna2 activity are processed by Holliday junction resolvase Yen1. *Nature Communications*, 7:13157.

Pachathundikandi, Suneesh Kumar; Müller, Anne; Backert, Steffen (2016). Inflammasome activation by helicobacter pylori and its implications for persistence and immunity. In: Backert, Steffen. *Inflammasome Signaling and Bacterial Infections*. Germany: Springer, 117-131.

Pierroz, Vanessa; Rubbiani, Riccardo; Gentili, Christian; Patra, Malay; Mari, Cristina; Gasser, Gilles; Ferrari, Stefano (2016). Dual mode of cell death upon the photo-irradiation of a Ru(II) polypyridyl complex in interphase or mitosis. *Chemical Science*, 7(9):6115-6124.

Pinto, Cosimo; Kasaciunaite, Kristina; Seidel, Ralf; Cejka, Petr (2016). Human DNA2 possesses a cryptic DNA unwinding activity that functionally integrates with BLM or WRN helicases. *eLife*, 5:e18574.

Piwko, Wojciech; Mlejnkova, Lucie J; Mutreja, Karun; Ranjha, Lepakshi; Stafa, Diana; Smirnov, Alexander; Brodersen, Mia M; Zellweger, Ralph; Sturzenegger, Andreas; Janscak, Pavel; Lopes, Massimo; Peter, Matthias; Cejka, Petr (2016). The MMS22L-TONSL heterodimer directly promotes RAD51-dependent recombination upon replication stress. *EMBO Journal Online*, 35:2584-2601.

Ribeyre, Cyril; Zellweger, Ralph; Chauvin, Maeva; Bec, Nicole; Larroque, Christian; Lopes, Massimo; Constantinou, Angelos (2016). Nascent DNA proteomics reveals a chromatin remodeler required for topoisomerase I loading at replication forks. *Cell Reports*, 15(2):300-309.

Saredi, Giulia; Huang, Hongda; Hammond, Colin M; Alabert, Constance; Bekker-Jensen, Simon; Forne, Ignasi; Reverón-Gómez, Nazaret; Foster, Benjamin M; Mlejnkova, Lucie; Bartke, Till; Cejka, Petr; Mailand, Niels; Imhof, Axel; Patel, Dinshaw J; Groth, Anja (2016). H4K20me0 marks post-replicative chromatin and recruits the TONSL-MMS22L DNA repair complex. *Nature*, 534(7609):714-718.

Smits, Hermelijn H; Hiemstra, Pieter S; Prazeres da Costa, Clarissa; Ege, Markus; Edwards, Michael; Garn, Holger; Howarth, Peter H; Jartti, Tuomas; de Jong, Esther C; Maizels, Rick M; Marsland, Ben J; McSorley, Henry J; Müller, Anne; Pfefferle, Petra I; Savelkoul, Huub; Schwarze, Jürgen; Unger, Wendy W J; von Mutius, Erika; Yazdanbakhsh, Maria; Taube, Christian (2016). Microbes and asthma: Opportunities for intervention. *Journal of Allergy and Clinical Immunology*, 137(3):690-697.

Urban, Vaclav; Dobrovolna, Jana; Hühn, Daniela; Fryzelkova, Jana; Bartek, Jiri; Janscak, Pavel (2016). RECQ5 helicase promotes resolution of conflicts between replication and transcription in human cells. *Journal of Cell Biology*, 214(4):401-415.

Dissertations

Anand, Roopesh. From resection to resolution : biochemical investigation of early and late steps of homologous recombination. 2016, University of Zurich, Faculty of Science.

Bolck, Hella A. A novel connection between CtIP and the BRCA1/BARD1 tumour suppressor complex emerges through functional RNAi screening. 2016, University of Zurich, Faculty of Science.

Di Marco, Stefano. Role of RECQ5 Helicase in MUS81-mediated Resolution of Aberrant Replication Intermediates. 2016, University of Zurich, Faculty of Science.

Falke, Martin. Second Generation Protein Replacement System to Study MLH1 Missense Mutations. 2016, University of Zurich, Faculty of Science.

Flori, Michael. The hematopoietic oncoprotein FOXP1 promotes cell survival in diffuse large B-cell lymphoma by repressing S1PR2 signaling. 2016, University of Zurich, Faculty of Science.

Lepakshi, Lepakshi. Role of *Saccharomyces cerevisiae* Mlh1-Mlh3 (MutLY) in Meiotic Homologous Recombination. 2016, University of Zurich, Faculty of Science.

Mlejnkova, Lucie Jarmila. Elucidating the Role of MMS22L-TONSL Heterodimer in Homologous Recombination. 2016, University of Zurich, Faculty of Science.

Pizzolato, Julia. Biochemical Characterization of FAN1 Nuclease. 2016, University of Zurich, Faculty of Science.

Sturzenegger, Andreas. Study of Mechanisms of Homologous Recombination in Mammalian Cells. 2016, University of Zurich, Faculty of Science.

Vujanovic, Marko. Mechanistic Insight Into Replication Fork Reversal Under Genotoxic Stress. 2016, University of Zurich, Faculty of Science.

Weller, Marie-Christine. The Contribution of PARP-1 and ELG1/ATAD5 to Genomic Stability. 2016, University of Zurich, Faculty of Science.

2017

Arnold, Isabelle C; Zhang, Xiaozhou; Urban, Sabine; Artola-Borán, Mariela; Manz, Markus G; Ottemann, Karen M; Müller, Anne (2017). NLRP3 Controls the Development of Gastrointestinal CD11b+ Dendritic Cells in the Steady State and during Chronic Bacterial Infection. *Cell Reports*, 21(13):3860-3872.

Balbo Pogliano, Chiara; Gatti, Marco; Rütthemann, Peter; Garajová, Zuzana; Penengo, Lorenza; Naegeli, Hanspeter (2017). ASH1L histone methyltransferase regulates the handoff between damage recognition factors in global-genome nucleotide excision repair. *Nature Communications*, 8(1):1333.

Boege, Yannick; Malehmir, Mohsen; Healy, Marc E; Wolf, Monika Julia; Böhm, Friederike; Frick, Lukas; Maire, Renaud; Moch, Holger; Heikenwalder, Mathias; Weber, Achim; Lopes, Massimo; et al (2017). A Dual Role of Caspase-8 in Triggering and Sensing Proliferation-Associated DNA Damage, a Key Determinant of Liver Cancer Development. *Cancer Cell*, 32(3):342-359.e10.

Di Marco, Stefano; Hasanova, Zdenka; Kanagaraj, Radhakrishnan; Chappidi, Nagaraja; Altmanova, Veronika; Menon, Shruti; Sedlackova, Hana; Langhoff, Jana; Surendranath, Kalpana; Hühn, Daniela; Bhowmick, Rahul; Marini, Victoria; Ferrari, Stefano; Hickson, Ian D; Krejci, Lumir; Janscak, Pavel (2017). RECQ5 Helicase Cooperates with MUS81 Endonuclease in Processing Stalled Replication Forks at Common Fragile Sites during Mitosis. *Molecular Cell*, 66(5):658-671.e8.

Duroc, Yann; Kumar, Rajeev; Ranjha, Lepakshi; Adam, Céline; Guérois, Raphaël; Md Muntaz, Khan; Marsolier-Kergoat, Marie-Claude; Dingli, Florent; Laureau, Raphaëlle; Loew, Damarys; Llorente, Bertrand; Charbonnier, Jean-Baptiste; Cejka, Petr; Borde, Valérie (2017). Concerted action of the MutLβ heterodimer and Mer3 helicase regulates the global extent of meiotic gene conversion. *eLife*, 6:e21900.

Graf, Urs; Casanova, Elisa A; Wyck, Sarah; Dalcher, Damian; Gatti, Marco; Volenweider, Eva; Okoniewski, Michal J; Weber, Fabienne A; Patel, Sameera S; Schmid, Marc W; Li, Jiwen; Sharif, Jafar; Wanner, Guido A; Koseki, Haruhiko; Wong, Jiemin; Pelczar, Pawel; Penengo, Lorenza; Santoro, Raffaella; Cinelli, Paolo (2017). Pramel7 mediates ground-state pluripotency through proteasomal-epigenetic combined pathways. *Nature Cell Biology*, 19(7):763-773.

Hashwah, Hind; Schmid, Corina A; Kasser, Sabrina; Bertram, Katrin; Stelling, Anna; Manz, Markus G; Müller, Anne (2017). Inactivation of CREBBP expands the germinal center B cell compartment, down-regulates MHCII expression and promotes DLBCL growth. *Proceedings of the National Academy of Sciences of the United States of America*, 114(36):9701-9706.

Henrich, Sarah Marie; Usadel, Clemens; Werwein, Eugen; Burdova, Kamila; Janscak, Pavel; Ferrari, Stefano; Hess, Daniel; Klemnauer, Karl-Heinz (2017). Interplay with the Mre11-Rad50-Nbs1 complex and phosphorylation by GSK3β implicate human B-Myb in DNA-damage signaling. *Scientific Reports*, 7:41663.

Juskevicius, Darius; Müller, Anne; Hashwah, Hind; Lundberg, Pontus; Tzankov, Alexander; Menter, Thomas (2017). Characterization of the mutational profile of 11 diffuse large B-cell lymphoma cell lines. *Leukemia & Lymphoma*:Epub ahead of print.

Kyburz, Andreas; Müller, Anne (2017). *Helicobacter pylori* and Extragastric Diseases. In: Tegtmeyer, Nicole; Backert, Steffen. *Molecular Pathogenesis and Signal Transduction by Helicobacter pylori*. Springer International Publishing: Springer, 325-347.

Kyburz, Andreas; Urban, Sabine; Altobelli, Aleksandra; Floess, Stefan; Huehn, Jochen; Cover, Timothy L; Müller, Anne (2017). *Helicobacter pylori* and its secreted immunomodulator VacA protect against anaphylaxis in experimental models of food allergy. *Clinical and Experimental Allergy*, 47(10):1331-1341.

Levikova, Maryna; Pinto, Cosimo; Cejka, Petr (2017). The motor activity of DNA2 functions as an ssDNA translocase to promote DNA end resection. *Genes and Development*, 31(5):493-502.

Mejías-Luque, Raquel; Zöller, Jessica; Anderl, Florian; Loew-Gil, Elena; Vieth, Michael; Adler, Thure; Engler, Daniela B; Urban, Sabine; Browning, Jeffrey L; Müller, Anne; Gerhard, Markus; Heikenwalder, Mathias (2017). Lymphotoxin β receptor signalling executes *Helicobacter pylori*-driven gastric inflammation in a T4SS-dependent manner. *Gut*, 66(8):1369-1381.

Mijic, Sofija; Zellweger, Ralph; Chappidi, Nagaraja; Berti, Matteo; Jacobs, Kurt; Mutreja, Karun; Ursich, Sebastian; Ray Chaudhuri, Arnab; Nussenzweig, Andre; Jancsak, Pavel; Lopes, Massimo (2017). Replication fork reversal triggers fork degradation in BRCA2-defective cells. *Nature Communications*, 8(1):859.

Mordasini, Vanessa; Ueda, Seigo; Aslandogmus, Roberta; Berger, Christoph; Gysin, Claudine; Hühn, Daniela; Sartori, Alessandro A; Bernasconi, Michele; Nadal, David (2017). Activation of ATR-Chk1 pathway facilitates EBV-mediated transformation of primary tonsillar B-cells. *OncoTarget*, 8(4):6461-6474.

Odermatt, Diana C; Gari, Kerstin (2017). The CIA targeting complex is highly regulated and provides two distinct binding sites for client iron-sulfur proteins. *Cell Reports*, 18(6):1434-1443.

Otto, Claudia; Spivak, Graciela; Aloisi, Claudia M.N; Menigatti, Mirco; Naegeli, Hanspeter; Hanawalt, Philip C; Tanasova, Marina; Sturla, Shana J (2017). Modulation of cytotoxicity by transcription-coupled nucleotide excision repair is independent of the requirement for bioactivation of acylfulvene. *Chemical Research in Toxicology*, 30(3):769-776.

Parrotta, Rossella; Okonska, Agata; Ronner, Manuel; Weder, Walter; Stahel, Rolf; Penengo, Lorenza; Felley-Bosco, Emanuela (2017). A novel BRCA1-associated protein-1 isoform affects response of mesothelioma cells to drugs impairing BRCA1-mediated DNA repair. *Journal of Thoracic Oncology*, 12(8):1309-1319.

Porro, Antonio; Berti, Matteo; Pizzolato, Julia; Bologna, Serena; Kaden, Svenja; Saxer, Anja; Ma, Yue; Nagasawa, Kazuo; Sartori, Alessandro A; Jiricny, Josef (2017). FAN1 interaction with ubiquitylated PCNA alleviates replication stress and preserves genomic integrity independently of BRCA2. *Nature Communications*, 8(1):1073.

Schwendener, Reto A; Schott, Herbert (2017). Liposome formulations of hydrophobic drugs. In: D'Souza, Gerard G M. *Liposomes, Methods and Protocols*, Second Edition. New York, NY, USA: Springer, 73-82.

Takizawa, Hitoshi; Fritsch, Kristin; Kovtonyuk, Larisa V; Saito, Yasuyuki; Yakkala, Chakradhar; Jacobs, Kurt; Ahuja, Akshay K; Lopes, Massimo; Hausmann, Annika; Hardt, Wolf-Dietrich; Gomariz, Álvaro; Nombela-Arrieta, César; Manz, Markus G (2017). Pathogen-induced TLR4-TRIF innate immune signaling in hematopoietic stem cells promotes proliferation but reduces competitive fitness. *Cell Stem Cell*, 21(2):225-240.e5.

Urban, Vaclav; Dobrovolna, Jana; Jancsak, Pavel (2017). Distinct functions of human RecQ helicases during DNA replication. *Biophysical Chemistry*, 225:20-26.

Uzozie, Anuli Christiana; Selevsek, Nathalie; Wahlander, Asa; Nanni, Paolo; Grossmann, Jonas; Weber, Achim; Buffoli, Federico; Marra, Giancarlo (2017). Targeted proteomics for multiplexed verification of markers of colorectal tumorigenesis. *Molecular & Cellular Proteomics*:1-48.

Vindigni, Alessandro; Lopes, Massimo (2017). Combining electron microscopy with single molecule DNA fiber approaches to study DNA replication dynamics. *Biophysical Chemistry*, 225:3-9.

Vujanovic, Marko; Krietsch, Jana; Raso, Maria Chiara; Terraneo, Nastassja; Zellweger, Ralph; Schmid, Jonas A; Tagliatalata, Angelo; Huang, Jen-Wei; Holland, Cory L; Zwicky, Katharina; Herrador, Raquel; Jacobs, Heinz; Cortez, David; Ciccia, Alberto; Penengo, Lorenza; Lopes, Massimo (2017). Replication Fork Slowing and Reversal upon DNA Damage Require PCNA Polyubiquitination and ZRANB3 DNA Translocase Activity. *Molecular Cell*, 67(5):882-890.e5.

Dissertations

Kallenberger, Lia. Interplay of DNA Metabolic Pathways Involved in the Regulation of Gene Expression and Genomic Rearrangements. 2017, University of Zurich, Faculty of Science.

Kyburz, Andreas. Exploiting the Intergenerational Immunomodulatory Properties of Helicobacter Pylori for the Treatment of Allergic Disorders. 2017, University of Zurich, Faculty of Science.

Mijic, Sofija. The Role of BRCA2 in the Maintenance of Genome Stability in Response to Replication Stress. 2017, University of Zurich, Faculty of Science.

Mutreja, Karun. Monitoring the Replication and Repair of Psoralen Induced Interstrand Crosslinks on Human Genomic DNA. 2017, University of Zurich, Faculty of Science.

Pinto, Cosimo. Functions of Human DNA2 and its Protein Partners in DNA End Resection. 2017, University of Zurich, Faculty of Science.

Urban, Sabine. The Role of Dendritic Cells and Macrophages in Helicobacter Pylori Induced Immunity and Tolerance. 2016, University of Zurich, Faculty of Science.

Zwicky, Katharina. Mechanistic and Structural Insights into Unusual Replication Intermediates in Mammalian Cells. 2017, University of Zurich, Faculty of Science.

AWARDS

Massimo Lopes won the WBW (Wilhelm Bernhard Workshop) Young investigator award 2017.

Hind Haswah won a poster prize at the 7th Cancer Network Zurich Retreat 2017 in Emmetten.

Isabelle Arnold won one of the Best Workshop Presentation Awards for her presentation at the World Immune Regulation Meeting (WIRM) 2017 in Davos.

Anna Stelling received the Charles Rodolphe Brupbacher Young Investigator Award 2017.

Petr Cejka received the Friedrich Miescher Award 2017.

TEACHING

Petr Cejka

Genome stability and molecular cancer research: biochemical approaches
Scientific writing for PhD Students

Stefano Ferrari

Beyond central dogma: epigenetics, non-coding RNA, protein post-translational modifications and human disease
DNA metabolism and cancer
Genome instability and molecular cancer research
PhD Course "Cancer Biology": CellBiology: 1. Cell Signalling: relaying sets of instructions; 2. Mitotic Signalling and Cancer Therapy
PhD Course "Cancer Biology": The Eukaryotic Cell Division Cycle
Center for Integrative Biology (CIBIO), University of Trento, Italy - Beyond the central dogma: epigenetics and protein post-translational modifications in human disease

Kerstin Gari

Genome stability and molecular cancer research: biochemical approaches
Cancer - from genesis to therapy
Scientific writing course for PhD Students
ETH Zurich - Causes and consequences of unstable genomes

Pavel Janscak

DNA metabolism and cancer
Genome instability and molecular cancer research: Biochemical approaches

Josef Jiricny

Cancer Biology Master Course

Massimo Lopes

Coordinator and teacher of two courses for Master students:
Genome instability and molecular cancer research, DNA metabolism and cancer
Coordinator of the Master program in cancer biology

Giancarlo Marra

Beyond central dogma: epigenetics, non-coding RNA, protein post-translational modifications and human disease
Cancer Epigenetics
Cancer - from Genesis to Therapy
DNA metabolism and cancer
Epigenetics
Genome instability and molecular cancer research
Mantelstudium: Cancer - From genesis to therapy
PhD Course "Cancer Biology"- Epigenetic changes in colon cancer
ETH Zurich - Advances in translational cancer research: "Colon Cancer"
University of Trento, Italy - Epigenetics

Anne Müller

Cancer and the Immune System, lecture series for Master students in Cancer Biology
Cancer Biology Master course for biology students
Clinical Cancer Research for PhD students in the Cancer biology program
Infectious agents: From molecular biology to disease
Journal Club Systems Biology, Functional Genomics
Scientific writing for PhD Students
Tumor immunology for Cancer Biology PhD students
Vorlesung Molekulare Zellbiologie
Karolinska-Institute, Stockholm, Sweden - Infectious diseases and Cancer, block course for post-graduate students

Lorenza Penengo

Cancer Epigenetics

Alessandro A. Sartori

DNA metabolism and cancer
Genome instability and molecular cancer research
Vorlesung Molekulare Zellbiologie I
Vorlesung Molekulare Zellbiologie II

CONFERENCES

2016

Petr Cejka

Initiation of DNA double-strand break repair in yeast and humans
Weatherall Institute of Molecular Medicine, University of Oxford, UK

Initiation of DNA double-strand break repair in yeast and humans
IFOM, Milano, Italy

Initiation of DNA double-strand break repair in yeast and humans
ETHZ, Zurich

Mechanisms of DNA end resection in yeast and humans
Abcam conference on Mechanisms of Genetic Recombination, Alicante, Spain

Mechanisms of homologous recombination
Biozentrum, Basel

Stefano Ferrari

CDC25 Inhibitors
Abbvie Face-to-Face Meeting, Bio-Technopark, Zurich

Chromosome missegregation associated with RUVBL1 deficiency
Swiss Meeting, Genome Stability and Chromatin Dynamics, Emmetten

Kerstin Gari

Iron-sulphur clusters in DNA replication and repair
Swiss Meeting on Genome Stability and Chromatin Dynamics, Emmetten

Iron-sulphur proteins and genome stability
COST – FeSBionet Conference, Patras, Greece

Pavel Jancsak

RECQ5 DNA helicase promotes MUS81-mediated processing of late replication intermediates in mitosis
3rd International Meeting on RECQ Helicases in Biology and Medicine: RECQ2016 - Partnering for Progress, Fred Hutchinson Cancer Research Center, Seattle, USA

RECQ5 DNA helicase promotes MUS81-mediated processing of late replication intermediates in mitosis
Swiss Meeting on Genome Stability and Chromatin Dynamics, Emmetten

RECQ5 DNA helicase promotes MUS81-mediated processing of late replication intermediates in mitosis
ICGEB conference - At the Intersection of DNA Replication and Genome Maintenance: from Mechanisms to Therapy, Trieste, Italy

Role of RECQ5 DNA helicase in the processing of stalled replication forks
BRIC-CEHA Seminar, University of Copenhagen, Denmark

Josef Jiricny

Multifaceted Mismatch Repair
Conference - Responses to DNA damage: from molecular mechanism to human disease, Egmond aan Zee, NL

The enigmatic FAN1
GRC on Mutagenesis, Girona, Spain

Massimo Lopes

Replication fork remodeling upon replication/transcription interference?
CNRS Jacques Monod Conference: Transcription-replication crosstalk and genome instability, Roscoff, France

Replication fork remodeling upon replication stress in cancer and stem cells
Cancer Research Center, Toulouse, France

Replication fork remodeling upon replication stress in cancer and stem cells
Danish Cancer Research Center, Copenhagen, Denmark

Replication fork remodeling upon replication stress in cancer and stem cells
FIRC Institute of Molecular Oncology (IFOM), Milan, Italy

Replication Fork Remodeling upon DNA Replication Stress
Abcam Meeting: Mechanisms of Recombination, Alicante, Spain

Replication fork remodeling upon replication stress in cancer and stem cells
Edward A. Doisy Research Center (DRC), St. Louis University, USA

Replication Fork Remodeling upon DNA Replication Stress
Gordon Research Conference: DNA damage, mutation and cancer, Ventura, USA

Anne Müller

Helicobacter and extragastric diseases
12th International Conference on Pathogenesis and Host response in Helicobacter infections, Lo-skolen, Helsingor, Denmark

The gut microbiota and allergic diseases. Helicobacter has protective potential against extragastric diseases
29th International meeting of the European Helicobacter and microbiota study group, Magdeburg, Germany

Helicobacter pylori in health and disease
Helmholtz-Zentrum für Infektion, Braunschweig, Germany

Helicobacter pylori in health and disease
Karolinska-Institutet, Stockholm, Sweden

Helicobacter pylori in health and disease
University of Heidelberg, Dept. of Microbiology and Immunology, Heidelberg, Germany

Helicobacter pylori in health and disease
University of Basel, Pharmazentrum, Basel

Helicobacter pylori: pathogen or symbiont?
Symposium on Infection, Inflammation and Cancer, Liestal

Immunomodulatory activity of H. pylori on dendritic cells and T-cells
Infectious Disease Immunology meets Molecular Microbiology, Erlangen, Germany

Post-transcriptional and epigenetic regulation of diffuse large B-cell lymphomagenesis
Swiss Society for Immunology, Montreux

Lorenza Penengo

Deciphering the role of non-canonical ubiquitination in DNA damage response and DNA replication
Swiss Meeting, Genome Stability and Chromatin Dynamics, Emmetten

Role of non-canonical Ubiquitination in DNA damage response and DNA replication
EMBO course - New approaches to study ubiquitin and ubiquitin-like modifications, Alghero, Italy

Role of non-canonical ubiquitination in DNA damage response and DNA replication
Dept. of Oncology, Oxford, UK

Alessandro A. Sartori

CtIP and Genome Stability: New Insights into the Regulation of DNA-end Resection
Centro Andaluz de Biología Molecular & Medicina Regenerativa (CABIMER), Sevilla, Spain

New insights and tools for understanding the complexity of CtIP protein-protein interactions in genome maintenance
42nd Conference of the European Radiation Research Society, Amsterdam, The Netherlands

New insights and tools for understanding the complexity of CtIP protein-protein interactions in genome maintenance
14th Biennial Meeting of the German Society for Research on DNA Repair (DGDR), University of Duisburg-Essen, Germany

Organizer
Swiss Meeting on Genome Stability and Chromatin Dynamics, Emmetten

Session Chair
10th Quinquennial Conference - Responses to DNA damage: from molecule to disease, Egmond aan Zee, The Netherlands

2017

Stefano Ferrari

Targeting kinases through inhibition of the dual specificity phosphatase CDC25
9th Inhibitors of Protein Kinases Conference, Warsaw, Poland

Pavel Jancsak

RECQ5 cooperates with MUS81-EME1 in the processing of stalled replication forks at common fragile sites during mitosis
2nd DNA Replication as a Source of DNA Damage Conference: From Molecules to Human Health, Rome, Italy

Massimo Lopes

Replication fork remodeling: a double-edged sword in genome stability and cancer therapy
IGBMC, Strasbourg France

Replication fork remodeling: a double-edged sword in genome stability and cancer therapy
Eukaryotic DNA Replication and Genome Maintenance meeting, Cold Spring Harbor Laboratory, USA

Replication fork remodeling: a double-edged sword in genome stability and cancer therapy
25th Wilhelm Bernhard Workshop on the cell nucleus, Nizhny Novgorod, Russia

Replication fork remodeling: a double-edged sword in genome stability and cancer therapy
Institute Curie, Orsay, France

Replication fork remodeling: a double-edged sword in genome stability and cancer therapy
CEMMA (Cellular and molecular mechanisms in aging), University of Ulm, Germany

Replication fork reversal triggers fork degradation in BRCA2-defective cells
EMBO Young Investigator Genome Stability, Sectoral meeting, Heraklion, Greece

Replication fork remodeling: a double-edged sword in genome stability and cancer therapy
KSBMB International Conference, Busan, Korea

Replication fork remodeling: a double-edged sword in genome stability and cancer therapy
Center for Genomic Integrity, Institute for Basic Science, Ulsan, Korea

Anne Müller

Division of labor among myeloid cells during H. pylori infections
European Midwinter Immunology meeting, St. Johann, Austria

Helicobacter pylori in health and disease
European Helicobacter Study Group meeting, Magdeburg, Germany

Helicobacter pylori in health and disease
Gordon Research Conference on Microbial Adhesion and Signal Transduction, Newport, RI, USA

Helicobacter pylori in health and disease
Swiss Institute for Allergy Research, Davos

Helicobacter pylori in health and disease
Workshop on Infectious diseases, MPI Infection Biology, Berlin, Germany

Molecular pathogenesis of diffuse large B-cell lymphoma: opportunities for personalized treatment strategies
Clinical Research Day, University Hospital Zurich, Zurich

Molecular pathogenesis of diffuse large B-cell lymphoma: opportunities for personalized treatment strategies
Cancer Network Zurich Retreat, Emmetten

Lorenza Penengo

Histone ubiquitination by the DNA damage response is required for efficient DNA replication in unperturbed S-phase
Genomic Instability Center, La laguna, Tenerife, Spain

A role for the RNF8/RNF168/53BP1 pathway in replication fork progression and remodelling
CSHL meeting, Eukaryotic DNA Replication & Genome Maintenance, Cold Spring Harbour, USA

Alessandro A. Sartori

CtIP, BRCA1 and BRCA2: DNA repair and beyond
Institute for Research in Biomedicine (IRB), Barcelona, Spain

Dissecting the role of CtIP in alleviating DNA replication stress
EMBO Conference: The DNA damage response in cell physiology and disease", Cape Sounia, Athens, Greece

My (vague) memories of being a postdoc in the Jackson lab
2nd Jackson Lab Reunion, Clare College, Cambridge, UK

FAN1 acts in concert with ubiquitylated PCNA to alleviate replication stress and maintain genome stability (Talk A. Porro)
Cancer Network Zurich (CNZ) Retreat, Emmetten

A BRCA2-derived cell-penetrating peptide inhibits RAD51-mediated repair of DNA double-strand breaks (Talk A. Trenner)
Cancer Network Zurich (CNZ) Retreat, Emmetten

Identification of a miniature Sae2/Ctp1/CtIP ortholog from *Paramecium tetraurelia* reveals a conserved DNA-binding motif (Talk J. Godau)
Louis-Jeantet /LS² Satellite Meeting - The genetic evolution of cancer, Geneva

COLLABORATIONS

Bangor University, Bangor, UK

Centro Nacional de Investigaciones Oncologicas, Madrid, Spain

CNRS Délégation Ile-de-France Sud , Gif-sur-Yvette, France

Cold Spring Harbor Laboratory (CSHL), Cold Spring Harbor, NY, US

Columbia University, New York, NY, US

Deutsches Krebsforschungszentrum, Heidelberg, Germany

Erasmus University of Rotterdam, Rotterdam, The Netherlands

ETH Zurich, Zurich

Friedrich Miescher Institute for Biomedical Research (FMI), Basel

Friedrich-Alexander-University, Erlangen-Nürnberg, Germany

Helmholtz Centre for Infection Research, Braunschweig, Germany

Institut Curie, Paris, France

Institute for Research in Biomedicine, Bellinzona

Institute of Oncology Research, Bellinzona

Istituto Superiore di Sanità (I.S.S.), Roma, Italy

IT University of Copenhagen, Copenhagen, Denmark

Kantonsspital St. Gallen, St. Gallen

Leiden University Medical Center, Leiden, The Netherlands

Ludwig-Maximilians-University, München, Germany

Masaryk University, Brno, Czech Republic

Ospedale di Cremona, Cremona, Italy

Paris Sciences et Lettres (PSL), Paris, France

Radboud University Nijmegen, Nijmegen, The Netherlands

Rheinische Friedrich-Wilhelms-University Bonn, Bonn, Germany

Stadtspital Triemli, Zurich

Stanford University, Stanford, CA, US

The Netherland Cancer Institute (NKI/AvL), Amsterdam, The Netherlands

Universitat Autònoma de Barcelona, Barcelona, Spain

University College London (UCL), London, UK

University of Aberdeen, Aberdeen, UK

University of Basel, Basel

University of Bern, Bern

University of California, Santa Cruz, CA, UK

University of Copenhagen, Copenhagen, Denmark

University of Dundee, Dundee, UK

University of Eastern Piedmont Amedeo Avogadro, Vercelli, Italy

University of Geneva, Geneva

University of Kyoto, Kyoto, Japan

University of La Laguna, La Laguna, Spain

University of Lausanne, Lausanne

University of Leiden, Leiden, The Netherlands

University of Milano-Bicocca, Milano, Italy

University of Padova, Padova, Italy

University of Santiago de Compostela, Santiago de Compostela, Spain

University of Sussex, Brighton, UK

University of Trento, Trento, Italy

University of Vienna, Vienna, Austria

University of Washington, Seattle, WA, US

Vanderbilt University, Nashville, TN, US

Westfälische Wilhelms-University, Münster, Germany

SEMINARS

2016

Kara Bernstein
School of Medicine University of Pittsburgh, USA
Dissecting the role of the Rad51 paralogs during double-strand break repair

Simon Boulton
The Francis Crick Institute, South Mimms, UK
The Function and Execution of RTEL1 activities at vertebrate telomeres

Keith Caldecott
Genome Damage and Stability Centre, University of Sussex, Brighton, UK
DNA Strand Break Repair and Human Genetic Disease

Jeremy Austin Daniel
Center for Protein Research, University of Copenhagen, Denmark
Decoding the functional domains of a multi-tasking protein
Fabrizio d'Adda di Fagagna
IFOM - The FIRC Institute of Molecular Oncology, Milan; IGM-CNR, Pavia, Italy
DNA damage response activation in cancer and ageing and the role of non coding RNA

Simona Fiorani
Senior Editor, Nature Communications
How to get published in Nature and its sister journals

Andrew Jackson
MRC Human Genetics Unit Institute of Genetics and Molecular Medicine
University of Edinburgh, UK
From Autoinflammation to Zika: linking genome stability to innate immunity

Zvi Livneh
Faculty of Biochemistry, Dept. of Biological Chemistry Weizmann Institute
of Science, Israel
An inherently mutagenic process that protects us from cancer – the story of
translesion DNA synthesis

Niels Mailand
The Novo Nordisk Foundation Center for Protein Research, University of Co-
penhagen, Denmark
From proteomic exploration of DNA repair to new genome stability main-
tenance factors

Peter McKinnon
St Jude Children's Research Hospital Memphis, USA
Integrity Matters: Genome Integrity and Neurologic Disease

Kyle Miller
University of Texas, USA
The DNA damage response: Chromatin and Cancer

Arnab Ray Chaudhuri
NIH, Bethesda, USA
Protecting replication forks - implications in synthetic viability and chemo-
resistance

Sven Rottenberg
Institute of Animal Pathology, University of Bern
The study of PARP inhibitor resistance using genetically engineered mouse
models for BRCA1/2-mutated breast cancer

John Rouse
MRC Protein Phosphorylation and Ubiquitylation Unit, University of Dundee,
UK
Roles of the Fan1 nuclease in promoting chromosomal stability and human
health

Nina R. Salama
Fred Hutchinson Cancer Research Center, Division of Human Biology, Seattle,
Washington, USA
Bacterial adaptations for life in the stomach

2017

Ross Chapman

Wellcome Trust Centre for Human Genetics, University of Oxford, UK
Molecular delineation of the 53BP1-dependent DNA repair system

Oskar Fernandez-Capetillo

CNIO, Madrid, Spain
Exploring the role of replicative stress on cancer and ageing

David Komander

MRC Laboratory, Cambridge, UK
Specificity in the ubiquitin system

Stephen Kowalczykowski

College of Biological Sciences, UC Davis, USA
Understanding Biology, One Molecule at a Time

Andreas Moor

Weizmann Institute of Science, Rehovot, Israel
Spatial division of labor: from the healthy intestinal epithelium to colorectal cancer

Francesco Neri

Leibniz Institute on Aging, Fritz Lipmann Institute (FLI), Jena, Germany
Functional role of intragenic DNA methylation

Boris Pfander

Max-Planck Institute of Biochemistry, Martinsried, Germany
Control of Homologous Recombination - Cell Cycle and Chromatin State

Simon Reed

Division of Cancer and Genetics, School of Medicine, Cardiff University, UK
How the Global Genome-NER complex promotes nucleosome remodelling throughout the genome in response to UV damage

Evi Soutoglou

IGBMC Illkirch, France
Nuclear compartmentalization of DNA repair

Claus Storgaard Sørensen

Biotech Research and Innovation Centre (BRIC) University of Copenhagen, Denmark
Novel DNA nuclease function governs cell fate decisions after ionising radiation

Haico van Attikum

Leiden University Medical Center, The Netherlands
Regulation and dynamics of DNA repair in a chromatin context

Marc van de Wetering

Hubrecht Institute and Princes Maxima Center for Pediatric Oncology, Utrecht, NL
Modelling cancer in organoids

Hartmut Vodermaier

Senior Editor at the EMBO Journal
Science Publishing: Making a Black Box Transparent

Achim Weber

Group of Experimental and Molecular Pathology, Institute of Pathology, USZ, Zurich
Liver cancer: Pathology and Pathogenesis

Thorsten Zenz

Division of Hematology, USZ, Zurich
Landscape of drug response phenotypes in leukemia and lymphoma

IMCR PERSONNEL



Principal Investigators

Petr Cejka*
Stefano Ferrari
Kerstin Gari
Pavel Janscak
Josef Jiricny*
Massimo Lopes
Giancarlo Marra
Anne Müller
Lorenza Penengo
Alessandro A. Sartori

Postdoctoral Research

Assistants

Isabelle Arnold
Matteo Berti
Elda Cannavó*
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Christian Gentili*
Sara Giovannini*
Dorothea Gruber*
Kurt Jacobs
Stanislaw Jozwiakowski
Saho Kobayashi*
Shunsuke Kobayashi*
Jana Krietsch
Maryna Levikova*
Antonio Porro

PhD Students

Aleksandra Altobelli
Roopesh Anand*
Hella Bolck*
Michael Bauer
Katrin Bertram
Naga Raja Chappidi
Giuseppe De Gregorio*
Stefano Di Marco*
Martin Falke*
Angela Fallegger
Lorenza Ferretti*
Michael Flori*
Hadi Gharibi*
Julia Godau
Hind Hashwah
Sarah Himmels
Sean Howard*
Esin Isik
Lia Kallenberger*
Katrin Koch*
Andreas Kyburz
Richard Lutz
Shruti Menon
Anca-Irina Mihai
Sofija Mijic
Lucie Mlejnkova*
Karun Mutreja
Diana Odermatt
Stephany Orjuela
Maria Dilia Palumbieri
Hannah Parker
Franziska Pfistner
Cosimo Pinto*
Julia Pizzolato*
Bartlomiej Porebski
Sara Przetocka
Lepakshi Ranjha*
Maria Chiara Raso
Corina Schmid*
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