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Institute of Molecular Cancer Research

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JOSEF JIRICNY

Director

FOREWORD

Since its conception almost 20 years ago, the IMCR has grown from 15 to more than 80 persons and its scientists have achieved a great deal, as documented also by the ERC grants awarded to Petr Cejka, Massimo Lopes, Anne Müller and myself. The Institute now has international recognition as one of the European strongholds of DNA repair and genomic instability. IMCR scientists are regularly invited to speak at international congresses and participate in joint programs of the European Community. I am particularly proud of the fact that our achievements are primarily the work of graduate students recruited into the Cancer Biology and Molecular Life Sciences PhD Programs of the Life Science Zurich Graduate School. I am confident that the current standing and performance of the IMCR will improve further in the future, in spite of its limited resources, due to the high quality and dedication of its research team and its well-functioning infrastructure.

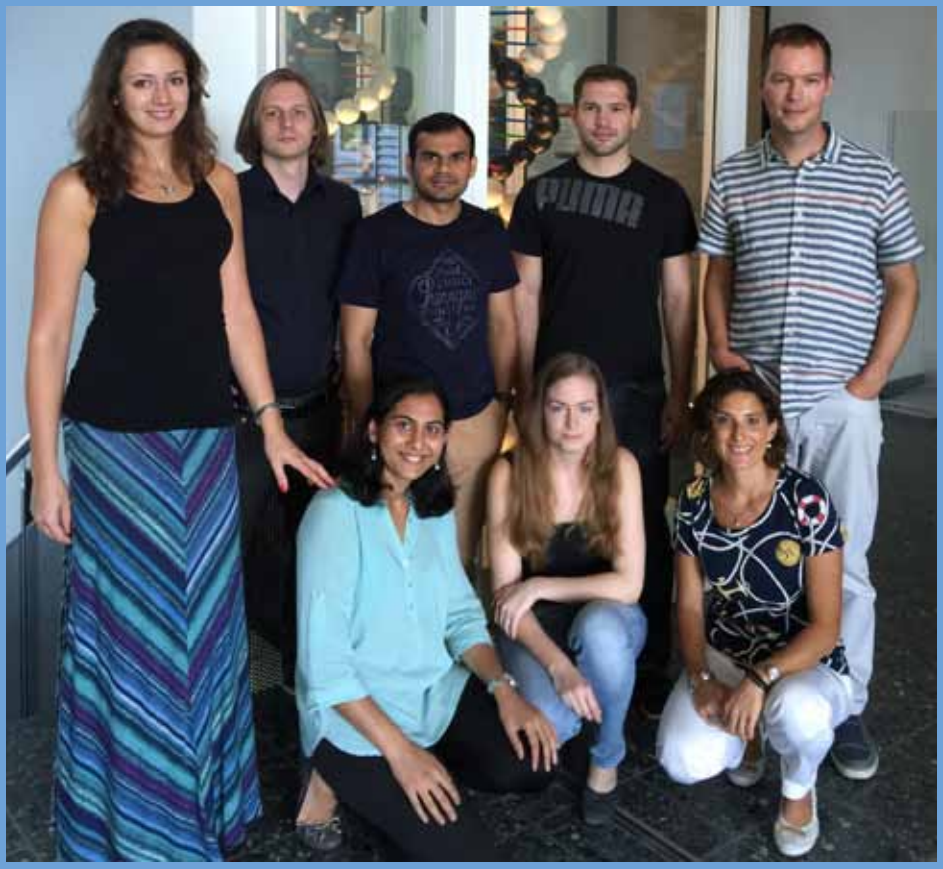
By optimizing laboratory and office space, we have been able to accommodate also the group of a new assistant professor, Lorenza Penengo, who joined us from the University of Piemonte Orientale. Lorenza will study ubiquitination in DNA damage response.

Congratulations are due to Massimo Lopes, who was promoted to associate professor ad personam and to Petr Cejka, who was awarded the Ernst-Theodor Jucker Prize for Cancer Research.

This is my last Scientific Report, as I shall be retiring in the summer of 2016. I am extremely proud of the IMCR; first and foremost of its academic achievements, but also of its spirit of friendliness, openness and collaboration. It has been a pleasure and a privilege for me to be able to spend two decades in the company of such talented young colleagues. I wish the institute a bright future.

PETR CEJKA

MECHANISMS OF HOMOLOGOUS RECOMBINATION



Postdocs
Elda Cannavó
Maryna Levikova

PhD Students
Roopesh Anand
Lepakshi
Lucie Mlejnkova
Cosimo Pinto

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.

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. The sources of DNA damage may stem from the environment, such as sunlight or chemicals, or result from regular cellular processes such as metabolism. These events represent a major challenge: if left unrepaired, the lesions could block access to the genetic information and prevent faithful replication (copying) of the DNA molecule. On the other hand, incorrect repair may lead to mutations (changes in genetic information) or chromosomal aberrations (larger scale rearrangements of DNA molecules). These events may threaten cell viability or, in some cases, result in uncontrolled cell division and cancer.

Throughout evolution, cells have evolved a number of DNA repair pathways that address different types 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. Unusually proficient DNA repair may lower treatment efficacy, while compromised DNA repair may lead to serious side effects.

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 will 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 that 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 without introducing mutations. Furthermore, 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 group is using both Saccharomyces cerevisiae and human systems.

We are using a combination of biochemical and genetic techniques. The biochemical approach involves the isolation of purified proteins, the active species that perform the molecular transactions of recombination. We are then studying the behavior of the recombinant proteins with specific DNA molecules in the test tube. Using this approach, we can learn much about the detailed molecular mechanism: what are the natural DNA substrates, how are they processed and what are the reaction outcomes. The genetic approach is then used to complement the biochemical studies. By specifically inactivating recombination genes or creating specific mutations that affect only a subset of functions, we can study the outcomes of recombination defects and interactions with related DNA metabolic pathways.

Our research group is interested, among others, in the following research topics.

Human MMS22L-TONSL complex: a link between DNA replication and homologous recombination

Lucie Mlejnkova

In addition to helping repair DNA breaks, homologous recombination helps to stabilize or restart replication forks upon DNA damage. 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 human MMS22L-TONSL complex might function in this process, but the underlying mechanism was unclear. We could show that MMS22L-TONSL heterodimer associates with RPA-coated single-stranded DNA, which likely recruits the complex to replication stall sites. MMS22L also directly interacts with the strand exchange protein RAD51. Importantly, recombinant MMS22L-TONSL limits the assembly RAD51 on dsDNA, which stimulates RAD51-ssDNA nucleoprotein filament formation and RAD51-dependent strand exchange activity in vitro. This provides an explanation of how this heterodimer helps maintain genome stability.

The function of human and yeast DNA2/Dna2 helicase-nuclease in DNA replication and repair

Maryna Levikova, Cosimo Pinto

Human DNA2 and yeast Dna2 are multifunctional enzymes that are involved in DNA replication, DNA repair by homologous recombination, long patch base excision repair, DNA damage signaling as well as telomere maintenance. These functions make Dna2 a key preserver of genome stability in eukaryotes. We determined that Dna2 can function in Okazaki fragment processing during DNA replication as a sole nuclease without the involvement of FEN1, in contrast to what has been believed previously. We also discovered that Dna2 possesses an unexpectedly vigorous DNA helicase activity. Surprisingly, this activity is cryptic in the wild type protein where it is masked by the Dna2 nuclease. Therefore, the helicase activity is only unleashed upon inactivation of this nuclease. We now have evidence that SUMOylation regulates the nuclease activity of Dna2 in vivo, which is required for proper function of Dna2. Finally, we study the helicase function of yeast and human Dna2/DNA2 variants, and how these integrate with the helicases Sgs1, BLM and WRN. This will help explain how these complexes function in the repair of DNA double-strand breaks.

Mechanistic insights into human and yeast MLH1-MLH3/Mlh1-Mlh3 proteins in meiotic recombination and mismatch repair

Lepakshi, Roopesh Anand, Nicolas Weyland

Meiosis is a specialized cellular division that forms gametes or spores. In this process, two chromosomes pair up and physically exchange segments of their genetic material. This occurs upon induction of double strand DNA breaks and subsequent formation of joint DNA molecule structures termed Holliday junctions, which are then processed by structure-specific nucleases to produce crossovers. Crossing over is important, because it generates progeny genomes that differ from those of the parents, contributing thus to genetic diversity. Recent genetic studies indicate that the Mlh1-Mlh3 (MutLγ) complex is responsible for the majority of crossovers in meiosis (80%). Its meiotic function is fully dependent on the integrity of its putative nuclease motif DQHA(X)2E(X)4E, inferring that its anticipated nuclease activity is involved in the processing of joint molecules to generate crossover recombination products. Additional factors including Msh4-Msh5, Exo1 and Sgs1 genetically and physically interact with the MutLγ complex and help produce crossovers. Despite the wealth of genetic and cell biological information about Mlh1-Mlh3, mechanistic insights into its function have been lacking due to the unavailability of the recombinant protein. How crossovers can be processed exclusively into crossovers remains a key question in the recombination field. We expressed and purified both yeast and human Mlh1-Mlh3 heterodimers and showed that they are indeed endonucleases as anticipated from genetic studies. The heterodimers also bind DNA with high affinity, and show a marked preference for Holliday junctions. The MSH4-MSH5 also binds HJs, and forms a ternary complex with MLH1-MLH3 on Holliday junctions. This work provides novel insights into the mode of Holliday junction recognition.

DNA end resection

Elda Cannavó

A long-standing interest of the Cejka laboratory is the elucidation of mechanisms underlying DNA end resection, which commits DSB repair into the recombination pathway. Specifically, resection of the 5′-terminated strand of the DSB produces a 3′ ssDNA overhang that serves as a template for the strand exchange protein Rad51 and primes DNA synthesis during later recombination steps. Using purified recombinant proteins, we could show that phosphorylated Sae2 activates a dormant dsDNA-specific endonuclease activity within the MRX complex, which is dependent on the integrity of the Mre11 nuclease active site. The endonuclease is specific to the 5′-terminated dsDNA strand, which explains the polarity paradox. The endonuclease of Mre11 requires a protein block in the vicinity of the broken DNA end. These findings complement previous genetic work and demonstrate a likely mechanism for the initiation of DSB processing in both vegetative and meiotic cells. We continue to study how this pathway is regulated by posttranslational modifications.

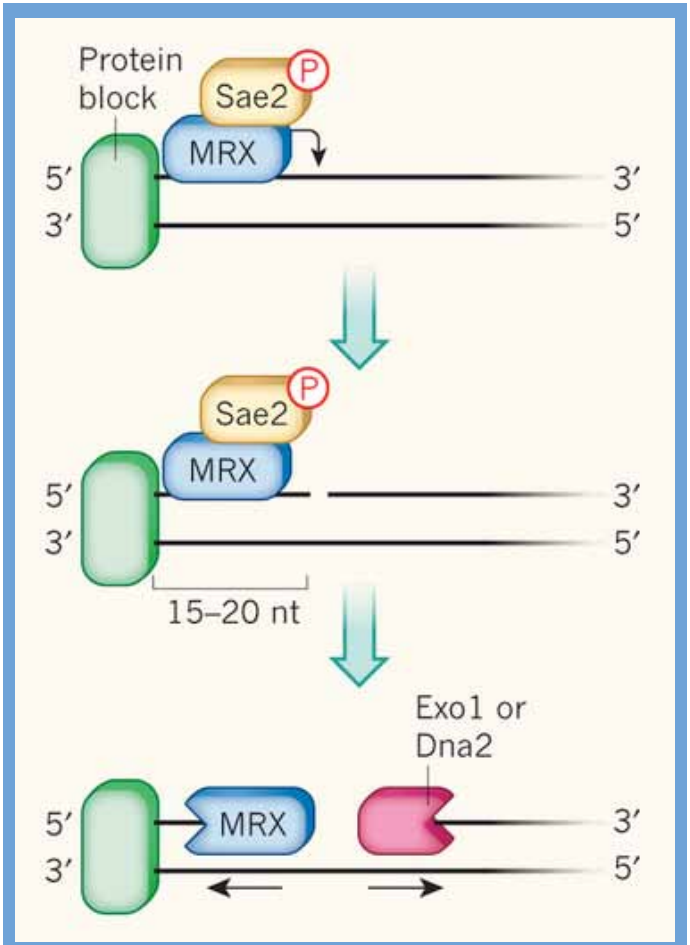


Figure.
Mre11-Rad50-Xrs2 proteins function together with phosphorylated Sae2 to initiate DNA double-strand break repair.
From Symington, 514(7520):39-40, 2014.

Funding
Swiss National Science Foundation, Swiss Cancer League

STEFANO FERRARI

DNA DAMAGE AND THE CELL CYCLE

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Christian Gentili

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Technician
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* left 2015

Faithful execution of the cell division cycle ensures correct transmission of genetic information to the progeny. Damage occurring to DNA during transition through the cell cycle is addressed before division, in order to maintain a stable genome and avoid the development of cancer. To this end, cells are equipped with dedicated protein complexes that orchestrate DNA repair while triggering checkpoints that arrest the cell cycle at defined transitions. Activation and suppression of checkpoints relies on rapid switches consisting of reversible post-translational modification (PTM) of proteins, such as phosphorylation, ubiquitination and SUMOylation.

The 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. In recent years, 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. The established adaptability of cancer cells to external insults, however, constantly demands the implementation of novel strategies. Basic research aims to satisfy this demand through the continuous discovery of novel targets.

On one front, my laboratory is devoting significant efforts to elucidate basic mechanisms governing the cellular response to DNA damage and how activation of DNA repair pathways influences the onset of mitosis. Aim of this work is the identification of novel checkpoint elements and the definition of synthetic lethality among genes, in the hope of identifying potential new therapeutic targets. On another front, we are engaged in the identification and characterization of metal complexes that could be employed as photosensitizers in photo-dynamic therapy of cancer (collaboration with Prof. G. Gasser, Univ. of Zurich).

Double-strand breaks (DSBs) are among the most cytotoxic lesions affecting DNA. They are generated by ionizing radiation (IR), certain chemotherapeutic drugs, collapse of stalled DNA replication forks or through physiological processes such as meiotic recombination. DSBs are addressed by error-prone Non-Homologous-End-Joining (NHEJ) or error-free Homologous-Directed-Repair (HDR) pathways, depending on the cell cycle phase in which the lesions occur. Processing DNA lesions in a manner suitable to error-free repair requires coordination and fine-tuning of the factors involved. Post-translational modifications (PTMs) represent a rapid and reversible means to control their function.

To shed light on the role of PTMs in the control of DNA end-resection at sites of damage, few years ago we decided to focus on a key factor of HDR pathways, the 5'-to-3' exonuclease-1 (EXO1). 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, and meiotic and mitotic recombination. Additionally, yeast Exo1 was shown to localize to stalled replication forks where it counteracts fork reversal.

Studies that we conducted in the past laid the foundation for our current work. Briefly, we provided evidence that, in response to stalled DNA replication, human EXO1 is regulated through phosphorylation-dependent poly-ubiquitination, which results in its proteasome-mediated degradation, whereas yeast Exo1 is regulated through interaction with 14-3-3 proteins. On the other hand, at DSBs, human EXO1 functionally interacts with CtIP/RBBP8 that modulates both EXO1 recruitment to DNA ends and its resection activity, thus contributing to the maintenance of genome stability.

14-3-3 proteins and DNA replication forks

Murat Aykut, Dilara Sahin, Giuseppe De Gregorio

Stalling and collapse of DNA replication forks is an important source of genome instability and has been implicated in early steps of carcinogenesis. Maintenance of stable intermediates as well as fork restart upon stalled replication requires the coordinated action of a number of proteins. We have previously shown that 14-3-3 proteins control yeast Exo1 to avoid untimely resection at and behind replication forks and this, in turn, contributes to maintain fork integrity. However, we obtained evidence that 14-3-3 proteins have additional roles in the control of fork restart that are independent of Exo1. In this study we identified Dpb3, one of DNA polymerase epsilon (Polε) accessory subunits, as novel interaction partner of 14-3-3 proteins. We demonstrated that *DPB3* deletion suppressed HU hypersensitivity of 14-3-3-deficient cells and facilitated fork restart. Deletion of *DPB3*, but not deletion of the other accessory subunit *DPB4,* was sufficient to accelerate DNA replication forks progression upon deoxynucleotide depletion by HU. We excluded variations in intracellular dNTP levels as well as a role for translesion polymerases in this effect on DNA replication dynamics. Based on the fact that accessory subunits were suggested to stabilize the interaction of Polε with dsDNA, our data suggest that lack of Dpb3 may cause a switch from processive to distributive DNA synthesis.

Control of human EXO1 by SUMOylation

Serena Bologna, Christian Gentili

In this study we focused on the elucidation of the molecular mechanism controlling EXO1 protein stability at stalled DNA replication forks. An RNAi-based screen of human E2-conjugating enzymes identified UBE2I, the human homolog of yeast Ubc9, as major effector of EXO1 stability. We provided evidence that EXO1 is SUMOylated in an UBC9-PIAS1/PIAS4-dependent manner *in vivo* and demonstrated conservation of this mechanism in yeast by the Ubc9-Siz1/Siz2 using an *in vitro* reconstituted system. Furthermore, we showed that EXO1 physically interacts with the de-SUMOylating enzyme SENP6 both *in vitro* and *in vivo*, in a manner that promotes EXO1 stability. Finally, we identified the major sites of SUMOylation in EXO1 and showed that ectopic expression of a SUMOylation-deficient form of EXO1 rescued the DNA damage-induced chromosomal aberrations observed in cells expressing wt-EXO1. Hence, this study identified a novel layer of regulation of EXO1 and allowed proposing a model according to which SUMO moieties mediate association of EXO1 with factors that help recruit it to stalled replication forks where its activity and stability are subsequently controlled by phosphorylation and ubiquitination (Fig. 1). Elucidation of pathways that regulate EXO1 function provides ideal targets for therapeutic intervention.

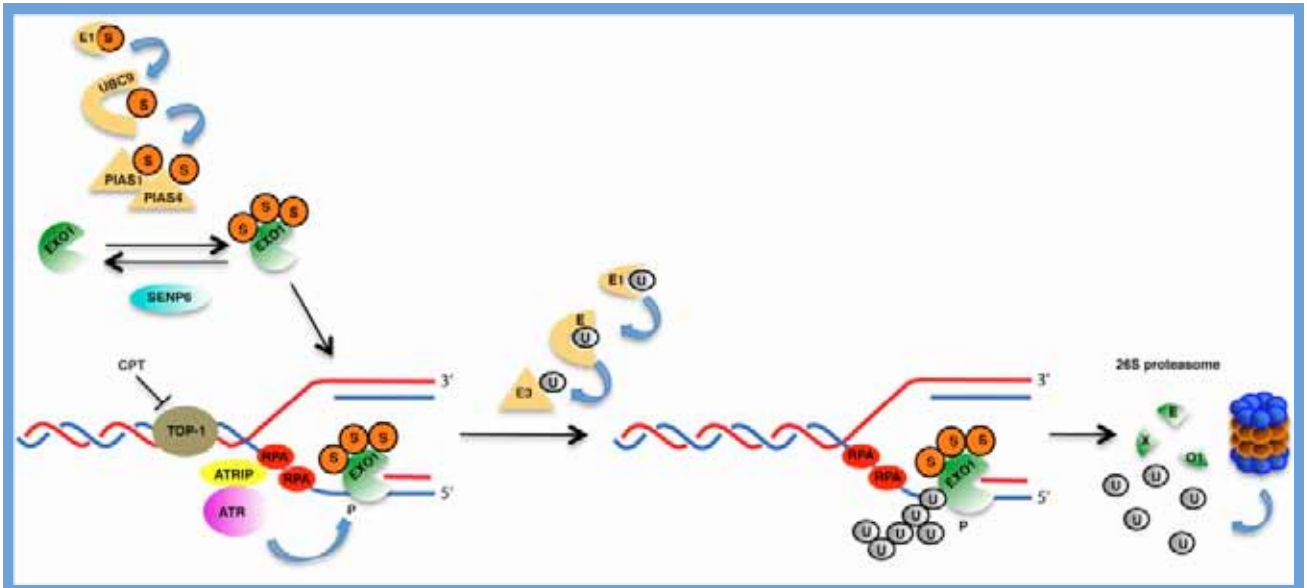

Figure 1.
PTMs controlling human EXO1 at stalled replication forks.
EXO1 SUMOylation is controlled by UBC9/SENP6. In response to stalled replication, EXO1 is recruited to forks where it promotes long-range resection of DNA ends. ATR-mediated phosphorylation facilitates EXO1 ubiquitination and degradation by 26S-proteasome pathways, avoiding pathological resection of DNA and contributing to maintain genome stability.

Control of yeast Exo1 by phosphorylation

Giuseppe De Gregorio

Evidence obtained in our and other laboratories indicated that *S. cerevisiae* Exo1 is controlled by protein interactions and reversible phosphorylation, but that yeast Exo1 protein stability is unaffected by DNA damage. In this study we set out to identify the kinase(s) and phosphatase(s) controlling Exo1 phosphorylation upon stalled DNA replication as well as the entire complement of phosphorylation sites in Exo1. The data obtained to date indicate that Exo1 phosphorylation in response to HU is Mec1/Rad53-dependent but Dun1-independent and that the protein phosphatases Glc7 and Pph3 are the most likely candidates for Exo1 dephosphorylation during checkpoint recovery (Fig. 2).

The low level of Exo1 expression in *S. cerevisiae* (estimated to ~800 molecules/cell) hampered our attempts to obtain extensive sequence coverage of the endogenous protein by mass spectrometry. Hence, considering that Rad53 is the only kinase of the Mec1 pathway able to phosphorylate Exo1 in response to stalled replication, we performed *in vitro* phosphorylation studies using Rad53 as kinase. Of the 14 sites identified, we focused on 5 residues that were phosphorylated at high stoichiometry. Functional studies showed that re-expression of Exo1-wt in exo1Δ sgs1Δ cells completely rescued growth in HU and to a good extent in CPT, whereas re-expression of Exo1-5A-Myc in the same background was unable to do so in HU and only partially in CPT. *In vivo* DNA resection assays that we are conducting in exo1Δ sgs1Δ strains will allow directly and unequivocally assessing the effect of phosphorylation on Exo1 activity.

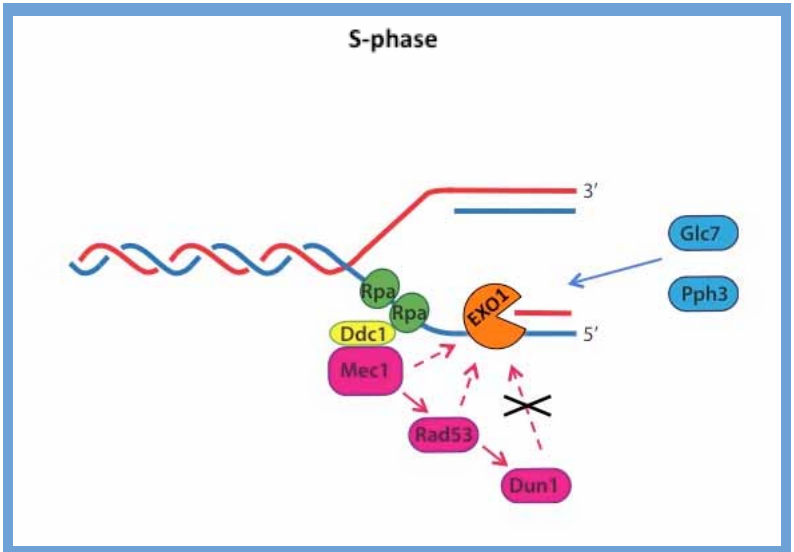

Figure 2 .
Phosphorylation-dependent control of yeast Exo1 at stalled replication forks. Upon stalled DNA replication, Exo1 is phosphorylated in a Mec1/Rad53-dependent but Dun1-independent manner at five major sites. During checkpoint recovery, Exo1 dephosphorylation is likely controlled by the protein phosphatases Glc7/Pph3.

Mitotic ATPases and genome stability

Christian Gentili

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 have been identified as components of the mismatch repairosome in the Jiricny laboratory. Interestingly, expression of both RUVBL1 and RUVBL2 is upregulated in cancer.

In this study we first examined localization of the RUVBL1/2 complex in mitotic cells. We observed that at anaphase-to-telophase transition, RUVBL1 localized to structures of the mitotic spindle apparatus, partially co-localizing with polo-like kinase 1 (PLK1). Interestingly, RUVBL1 and RUVBL2 separated during cytokinesis, with RUVBL1 remaining co-localized with PLK1. We obtained evidence that PLK1 is able to phosphorylate RUVBL1 - but not RUVBL2 - *in vitro* and we identified a single site of phosphorylation in the protein (T_{239}). This, along with the evidence of physical association between RUVBL1 and PLK1 *in vivo*, suggests that the latter may differentially regulate the function of the RuvB-like proteins during mitosis. Regarding the function of RUVBL1/2 in mitosis, we obtained evidence that siRNA-mediated knock-down of RuvB-like proteins leads to severe defects in chromosome alignment and segregation. More precisely, we observed that depletion of RUVBL1 or RUVBL2 expression caused extension of metaphase and delay of anaphase onset, with polar chromosomes failing to align at the metaphase plate and ultimately lagging at anaphase. In addition, we could show that the ATPase activity of RUVBL1 is indispensable for cell proliferation.

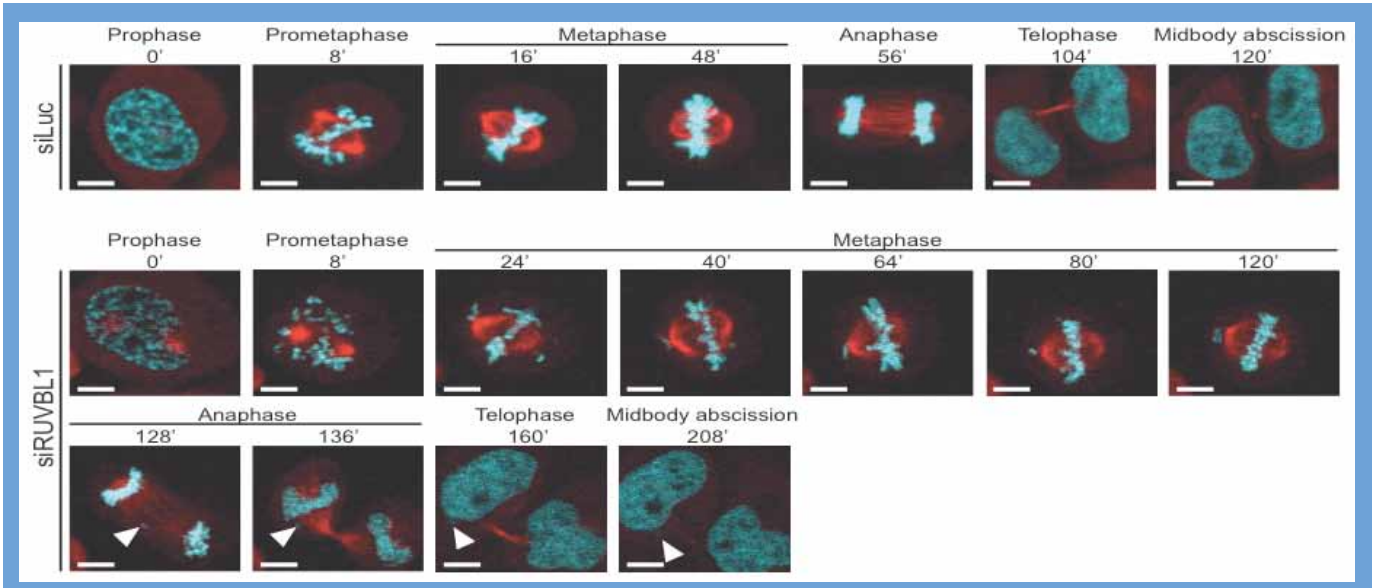

Figure 3.
RUVBL1 depletion affects the length of mitosis and results in lagging chromosomes.
RNAi-mediated knock-down studies followed by confocal live imaging of HeLa cells. Stills of control (Luc) or RUVBL1 siRNA-treated cells are shown. Lagging chromosomes are indicated with arrowheads. DNA is shown in cyan and α-tubulin in red.

Photoactivatable metal-based complexes and cancer therapy

Vanessa Pierroz, Christian Gentili (collaboration with Prof. G. Gasser)

Photodynamic therapy (PDT) is an attractive alternative to chemotherapy. Among the different photosensitizers (PSs) employed, Ru(II) polypyridyl complexes were found to be valid substitutes to porphyrin-based or phtalocyanine-based PSs. In this study we obtained evidence that one such complex, namely $[Ru(bipy)_2$-dppz-7-methoxy$][PF_6]_2$ (Ru65), localized in the nucleus of various cancer and normal cells and displayed cytotoxicity only upon UV-A irradiation. Importantly, we demonstrated that Ru65 intercalated in DNA and, upon light irradiation, promoted guanine oxidation, resulting in nicks in the double helix. We confirmed this mechanism of action in living cells showing that UV-A irradiation of cells loaded with Ru65 resulted in a transient DNA damage response that was rapidly extinguished. Strikingly, photo-irradiation of Ru65 triggered distinct mechanisms of cell death in interphase or mitotic cells. Interphase cells underwent cell cycle arrest at the G2/M phase and massive cytoplasmic vacuolation, which was paralleled by an unfolded-protein stress-response, resulting in reduction of viability and cell death through a paraptosis-like mechanism. On the other hand, UV-A irradiation of Ru65 in G2/M synchronized cells led to block of mitotic entry and rapid cell death through classic apoptotic pathways. Importantly, targeting mitotic cells with Ru65 allowed to significantly increase its photo-toxicity. Overall, our findings allow to draw two important conclusions: (i) PSs targeting the nucleus and causing a transient DNA damage response upon photoactivation represent an excellent alternative to current chemotherapeutics; (ii) combination of cell cycle inhibitors and PSs appears to be an even better alternative to the use of PSs alone for an effective clearance of cancer cells.

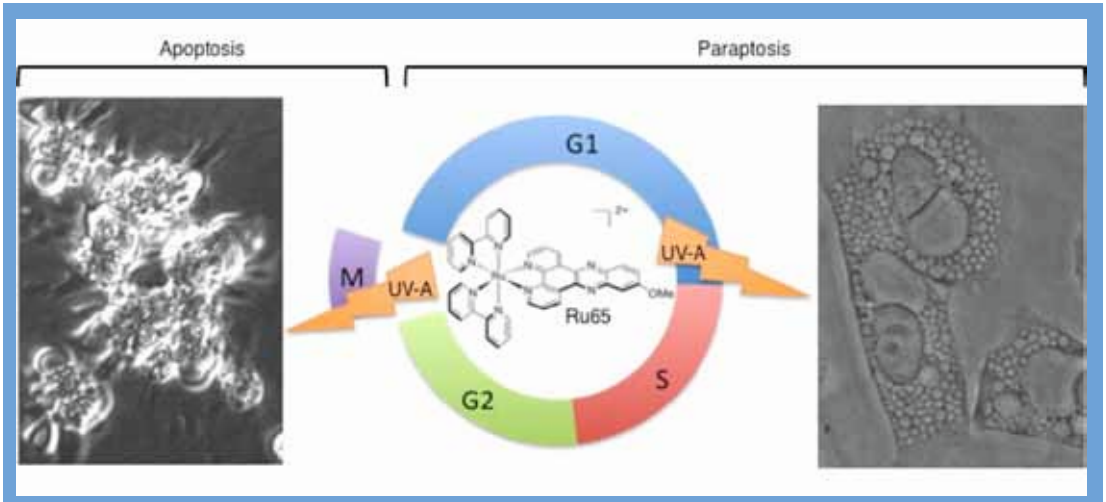

Figure 4.
Dual mode of cell death upon photo-irradiation of Ru65 in interphase and mitosis.
Scheme of the different cell death modes triggered by UV-A irradiation of Ru65 in interphase or at mitosis.

Funding
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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 that 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.

Maturation of FeS proteins

Diana Odermatt

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.

The maturation of nuclear FeS proteins takes place primarily in the cytoplasm, and is carried out by the cytoplasmic iron-sulphur assembly (CIA) machinery. We and others have shown that the HEAT-repeat protein MMS19, together with its interaction partner MIP18, plays a key role in this process by physically linking the CIA machinery and FeS target proteins. By doing so, it facilitates the transfer of an FeS cluster to an FeS apoprotein and allows it to become a fully functional FeS holoprotein (Figure 1A). In the absence of MMS19, when FeS protein maturation is impaired, the stability of FeS proteins and their assembly into functional complexes is affected (Figure 1B).
We are currently investigating the assembly of the so-called FeS targeting complex (MMS19–MIP18–CIAO1), and how it can mediate binding to many different FeS apoproteins that do not display any obvious sequence homology.

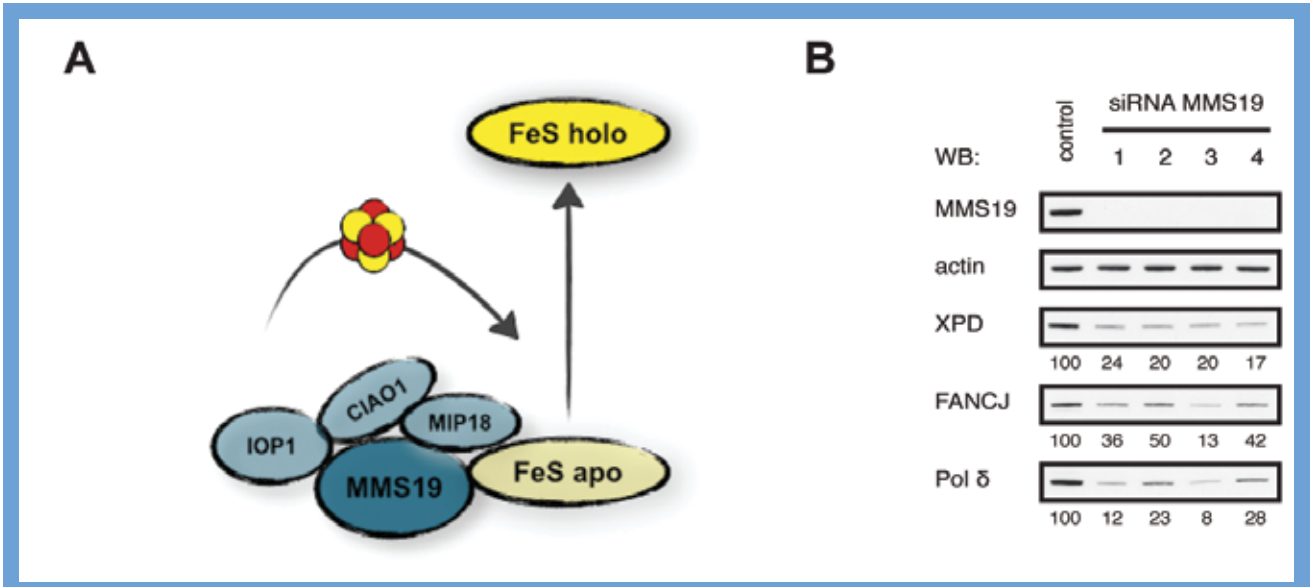

Figure 1.
(A) MMS19-MIP18 links cytoplasmic iron-sulphur cluster assembly to DNA metabolism.
(B) FeS protein stability is affected in the absence of MMS19 (from Gari et al., Science 2012).

Iron-sulphur clusters in DNA replication and repair proteins

Anna Simon, Richard Lutz, Stanislaw Jozwiakowski

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, FANCJ and ChlR1, 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 2). 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. 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 adaption 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.

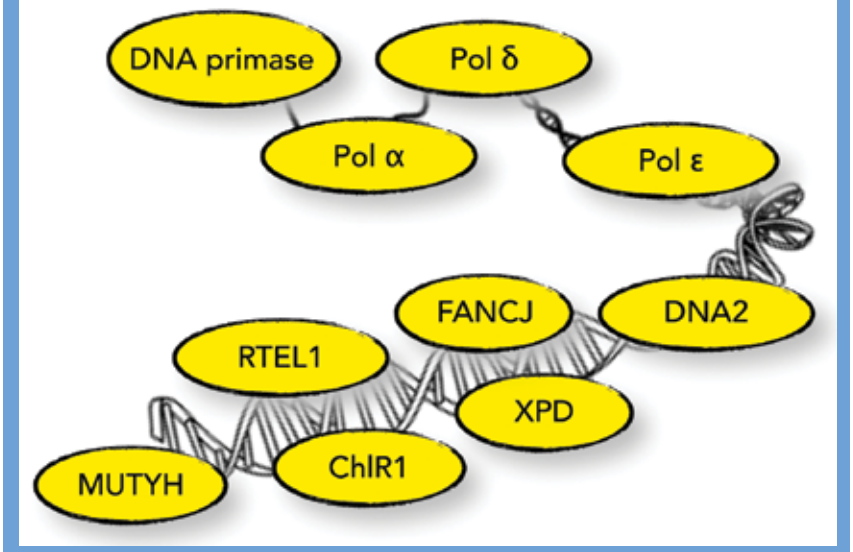

Figure 2. FeS proteins involved in the maintenance of genome stability.

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PAVEL JANSCAK

MECHANISMS OF DNA REPAIR

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Technician
Christiane König

The maintenance of genome integrity and fidelity is essential for the proper functioning and survival of all organisms. The research in our laboratory is centered on defining the molecular mechanisms underlying the DNA repair processes in human cells. A long-term goal of our work is to exploit this knowledge for the development of new therapeutic strategies for the treatment of cancer, which are based on targeting specific DNA-repair pathways with small molecule inhibitors.

Sensing and signaling of DNA damage

Andreas Sturzenegger, Naga Raja Chappidi

DNA damage triggers a complex network of pathways that sense and signal problems in the DNA, arrest cell-cycle progression and activate appropriate DNA-repair mechanisms. The key regulators of the mammalian DNA damage response are the ATM (ataxia telangiectasia mutated) and ATR (ATM and Rad3-related) protein kinases. ATM is the primary mediator of cellular response to DNA double-strand breaks (DSBs), while ATR has a crucial role in stabilizing the genome during DNA replication. ATR-mediated suppression of dormant replication origins shields active forks against irreversible breakage via preventing exhaustion of nuclear RPA that contributes to fork stabilization. We aim to gain a thorough understanding of the molecular mechanisms underlying the activation of ATR kinase in response to replication stress. Recent studied in our laboratory have identified the mismatch-binding protein MutSβ as a new DNA damage sensor in the process of ATR activation by replication-associated DSBs. Our work has demonstrated that MutSβ binds to hairpin loops persisting in RPA-coated single-stranded DNA (ssDNA) at sites of DNA damage and mediates the recruitment of the ATR-ATRIP complex, a prerequisite for ATR activation by TOPBP1. Based on these findings, we have proposed a model wherein the formation of hairpin loops in ssDNA generated at sites of DNA damage could signal lack of free RPA in the cell and serve as a trigger for ATR activation in a process mediated by MutSβ (Figure 1). Our current studies aim to gain further insight into the molecular mechanism of MutSβ-dependent ATR activation. Given synthetic lethal interaction between ATR and the ATM-p53 tumor suppressor pathway, we also aim to explore the possibility of using ATM inhibitors for specific killing of colorectal cancers associated with loss of MutSβ.

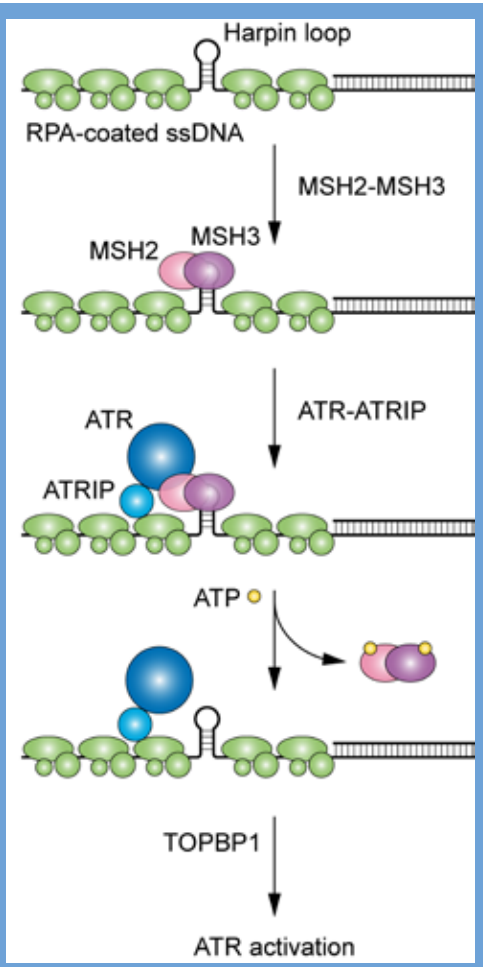

Figure 1.
Model for the role of MutSβ in ATR activation. MutSβ (MSH2–MSH3) binds to hairpin loop structures formed in RPA-coated ssDNA at sites of DNA damage and recruits the ATR-ATRIP complex. Subsequently, ATR-ATRIP binds directly to RPA-ssDNA leading to ATR activation by TOPBP1. ATP binding by MutSβ triggers its dissociation from ssDNA and the ATR-ATRIP complex.

Repair of DNA double-strand breaks by homologous recombination

Andreas Sturzenegger

We have a long-standing interest in understanding the biochemistry of DSB repair by homologous recombination (HR). HR, which is only operative during S/G2 phases of the cell cycle, is initiated by nucleolytic resection of broken DNA ends to generate 3'-ssDNA tails. One of these ssDNA tails is utilized for RAD51-mediated homology search on the undamaged sister chromatid, and after invasion and pairing with a homologous region, it primes DNA synthesis to restore the DNA continuity at the break site. Majority of HR events in mitotic cells proceed via the so-called synthesis-dependent strand annealing (SDSA) pathway, where the extended joined DNA molecule (D-loop) is disrupted by specialized DNA helicases and the newly synthesized DNA is annealed to the ssDNA tail of the other part of the broken chromosome, followed by DNA flap removal and ligation. However, HR has a second branch where the ssDNA tail of the non-invading DNA end is annealed to the D-loop to form a double Holliday junction (DHJ), whose resolution can lead to exchanges of the flanking DNA sequences between the donor and acceptor DNA molecules, the so-called crossovers. Work in our laboratory provided insights into the molecular mechanism underlying the preferential use of the SDSA pathway. Specifically, we have identified RECQ5 DNA helicase as a factor that acts during the post-synaptic phase of SDSA to prevent formation of aberrant RAD51 filaments on the extended invading strand, thus limiting its channeling into the potentially hazardous crossover pathway. In addition, we have investigated the biochemical mechanism of DSB-end resection in human cells. Studies in yeast have demonstrated that DNA-end resection during HR is mediated by either of the two nucleases: Exonuclease 1 (Exo1) and Dna2. Dna2 acts as a ssDNA-specific endonuclease and hence requires a DNA helicase to open the DNA duplex. Work conducted in our laboratory has revealed that human DNA2 catalyzes long-range DNA-end resection in conjunction with either WRN or BLM, both of which belong to the RecQ family of DNA helicases (Figure 2). Moreover, we have demonstrated that BLM mediates DNA-end resection as part of the BLM-TOPOIIIα-RMI1-RMI2 complex that is also involved in DHJ dissolution (Figure 2).

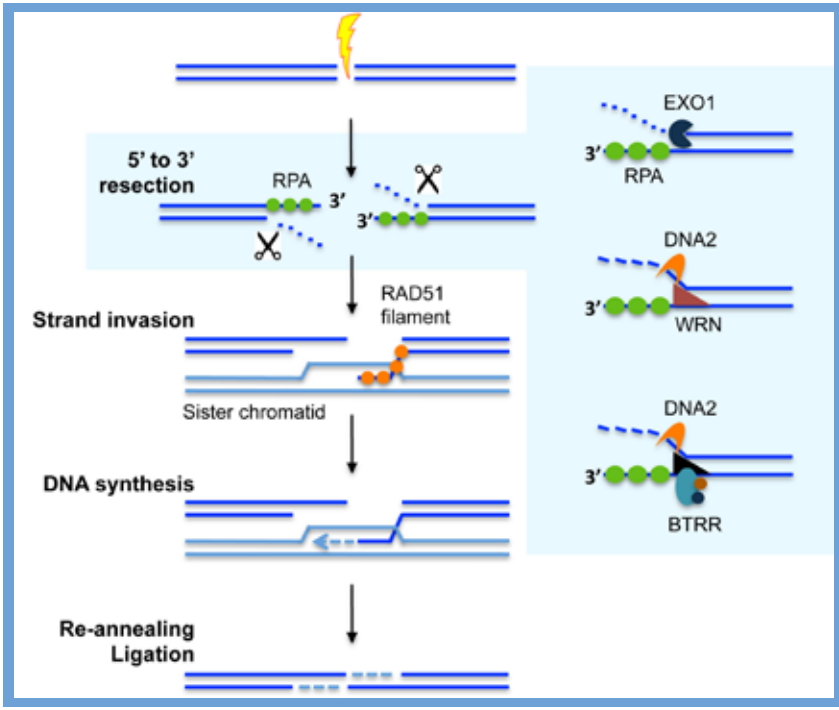

Figure 2.
Scheme of homologous recombination pathway for repairing DNA double-strand breaks.
Long-range DNA-end resection is mediated by EXO1 or DNA2 nucleases. DNA2 acts in conjunction with WRN or the BLM-TOPOIIIα-RMI1-RMI2 (BTRR) complex.

Processing of stalled replication forks

Stefano Di Marco, Naga Raja Chappidi

The progression of replication forks is frequently impaired by various physical obstacles such as unrepaired DNA lesions, active transcription complexes or secondary DNA structures, and slows down globally upon activation of oncogenes that deregulate the replication process. Replication fork stalling can have serious implications for genome stability and cell survival. Unreplicated DNA regions hamper proper chromosome segregation and can cause DNA damage in mitosis, which can lead to chromosomal rearrangements in the following G1 phase. Some genomic regions are particularly difficult to replicate and are unstable under conditions of replication stress. Most prominent amongst these are the so-called common fragile sites (CFSs), late-replicating regions that are frequently damaged in precancerous lesions and coincide with sites of recurrent chromosomal translocations found in cancers. One protein that plays a key role in the processing of stalled replication forks is the MUS81-EME1 endonuclease. Interestingly, we have found that MUS81 forms a stable complex with RECQ5 DNA helicase in human cells. Moreover, our work has revealed that RECQ5 binds directly to MUS81 and stimulates cleavage of forked DNA structures by MUS81-EME1 in vitro. More recently, we have obtained several lines of evidence suggesting that RECQ5 cooperates with MUS81-EME1 in the processing of late replication intermediates at CFSs during early mitosis to facilitate faithful chromosome segregation (Figure 3). Our ongoing studies aim to explore the role of RECQ5 in MUS81-mediated restart of stalled replication forks during S-phase.

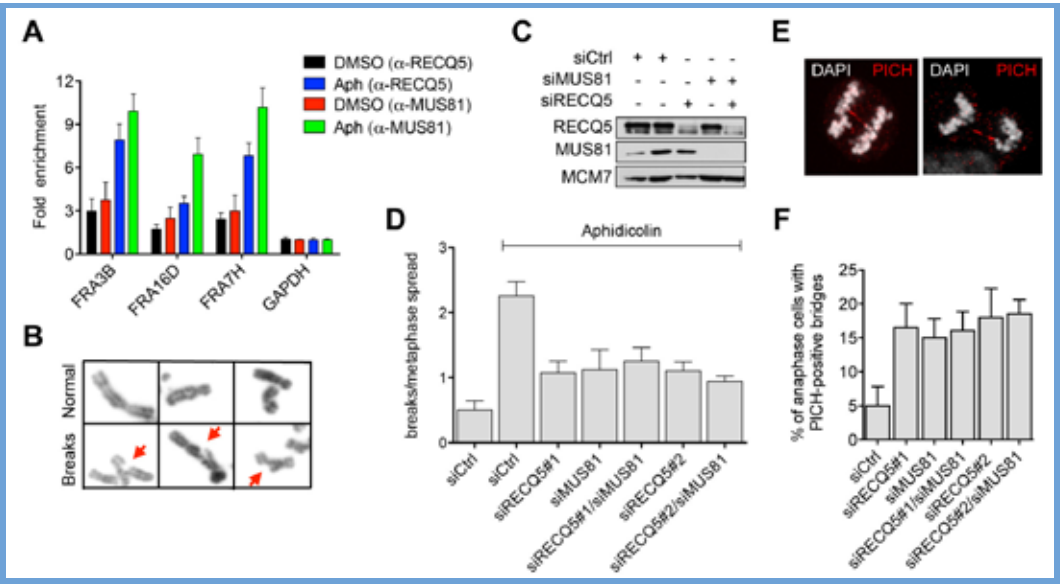

Figure 3. RECQ5 DNA helicase associates with common fragile sites and promotes their processing by MUS81 endonuclease in early mitosis. (A) Specific binding of RECQ5 and MUS81 to common fragile sites in response to replication stress. U2OS cells were treated with 0.2 μM aphidicolin (Aph) or DMSO for 16 h. Chromatin immunoprecipitation analysis coupled with quantitative PCR was performed using primers for three CFSs (FRA3B, FRA16D and FRA7H) and one control locus (GAPDH). (B) Examples of intact and broken metaphase chromosomes of U2OS cells treated with 0.2 μM Aph for 16 h. Arrows denote chromatid breaks. (C) Western blot analysis of extracts from U2OS cells transfected for 3 days with indicated siRNAs. siCtrl, control siRNA. (D) Quantification of fragile site expression in U2OS cells transfected with indicated siRNAs. Aph (or DMSO) was added 2 days after siRNA transfection. (E) Examples of anaphase cells with PICH-positive bridges. U2OS cells synchronized at G2/M transition by RO-3306 treatment (16 h) were released into mitosis for 1.5 hours before fixation and immunostaining with anti-PICH antibody. (F) Frequency of PICH-positive anaphase bridges in cells transfected with indicated siRNAs.

Molecular basis of transcription-associated genomic instability

Shruti Menon

DNA can be damaged during transcription if the nascent transcript pairs with the template DNA strand behind the transcription complex forming a three-stranded structure called an R-loop. In this aberrant structure, the non-transcribed DNA strand is left exposed as an extended ssDNA, which makes DNA bases in this strand more prone to chemical modifications and formation of secondary DNA structures that can compromise the progression of replication machinery. Formation of R-loops is facilitated by negative supercoiling generated behind the transcription complex and is favored in the transcriptional units containing runs of Gs in the non-transcribed strand. Recent studies provided evidence that R-loops form as a consequence of collisions between transcription and replication complexes, particularly if the progression of replication forks is perturbed. In addition, it has been hypothesized that formation of R-loops is the major source of genomic instability caused by oncogene-induced replication stress. We aim to identify the loci that are prone to R-loop formation upon replication stress. As a tool for the detection of R-loops, we generated a cell line conditionally expressing a catalytically-inactive form of RNase H1 fused to green fluorescent protein [RNH1(D210N)-GFP]. This RNaseH1 mutant stably binds RNA:DNA duplexes in R-loops, but does not cleave them. Our preliminary experiments have shown that RNH1(D210N)-GFP forms discrete nuclear foci in cells exposed to replication stress, indicative of R-loop formation (Figure 4). Ongoing work in the laboratory uses this tagged RNase H1 mutant to isolate by affinity chromatography R-loops from fragmented chromatin of cells exposed to replication stress for analysis of their DNA sequence by Illumina HiSeq sequencing. It is our keen interest to identify the proteins associated with R-loops by mass spectrometry and explore their roles in the maintenance of genomic stability.

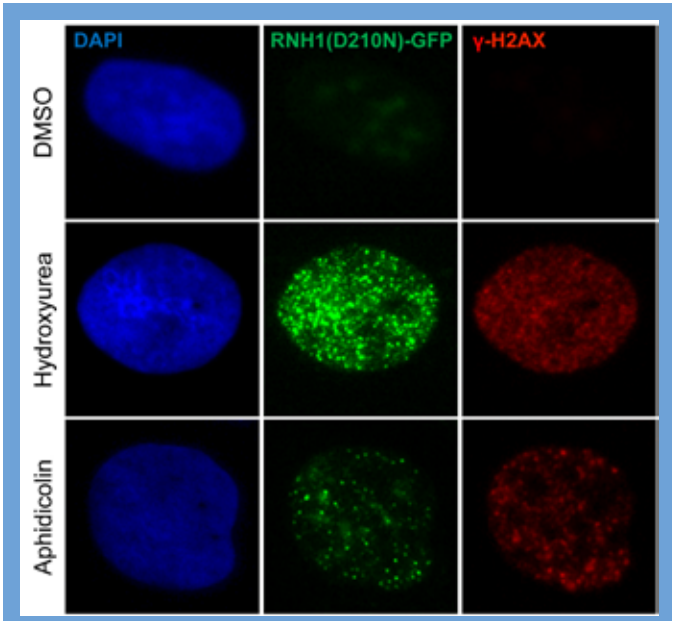

Figure 4.
Formation of nuclear foci of GFP-tagged RNase H1 (D210N) in response to replication stress. Expression of RNH1(D210N)-GFP in U2OS-T-REx cells was induced by doxycycline for 24 hours. For the last 6 hours, cells were treated with 10 mM hydroxyurea or, for the last 16 hours, with 0.2 μM aphidicolin. Control cells were treated with DMSO. After pre-extraction with PBS supplemented with 0.5% Triton X-100, cells were fixed with formaldehyde, immunostained for γ-H2AX and analyzed by fluorescence microscopy.

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JOSEF JIRICNY

THE MULTIFACETED MISMATCH REPAIR

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During the past two 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, but 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, or extracts of *Xenopus laevis* eggs. We are also revisiting the field of DNA methylation/demethylation, with a specific focus on the molecular mechanisms of these processes.

Biochemistry of mismatch repair I

Simone Repmann, Katja Kratz, Mariela Artola-Borán

To improve replication fidelity, MMR must detect non-Watson-Crick base pairs and excise them from the nascent DNA strand. It has been proposed that eukaryotic MMR distinguishes the nascent from the template strand by the presence of transient discontinuities, such as gaps between Okazaki fragments in the lagging strand, or the 3' terminus of the leading strand. Detection of a mismatch by the MSH2/MSH6 heterodimer (MutSα) activates a cryptic endonuclease in the MLH1/PMS2 (MutLα) heterodimer, which introduces additional nicks into the nascent strand and helps load EXO1, the exonuclease that degrades the error-containing strand towards and past the mismatch. The resulting single-stranded gap is then filled-in by one of the replicative polymerases. The repair process is completed by DNA ligase I, which seals the remaining nick. We wondered whether DNA breaks generated by other processes of DNA metabolism could be used by the MMR system as strand discrimination signals. We were able to show that transient breaks generated by uracil glycosylase-initiated base excision repair (Schantz et al., PNAS, 2009) or by RNaseH2 during the removal of ribonucleotides misincorporated into DNA (Ghodgaonkar et al., Mol. Cell, 2013) can act as initiation sites for MMR in human and yeast cells. In an attempt to learn whether this phenomenon was more general, we examined also other potential substrates. As shown in Figure 1, intermediates of oxidative damage processing by the base excision repair (BER) system could also be shown to be hijacked by the MMR system for the purposes of strand discrimination.

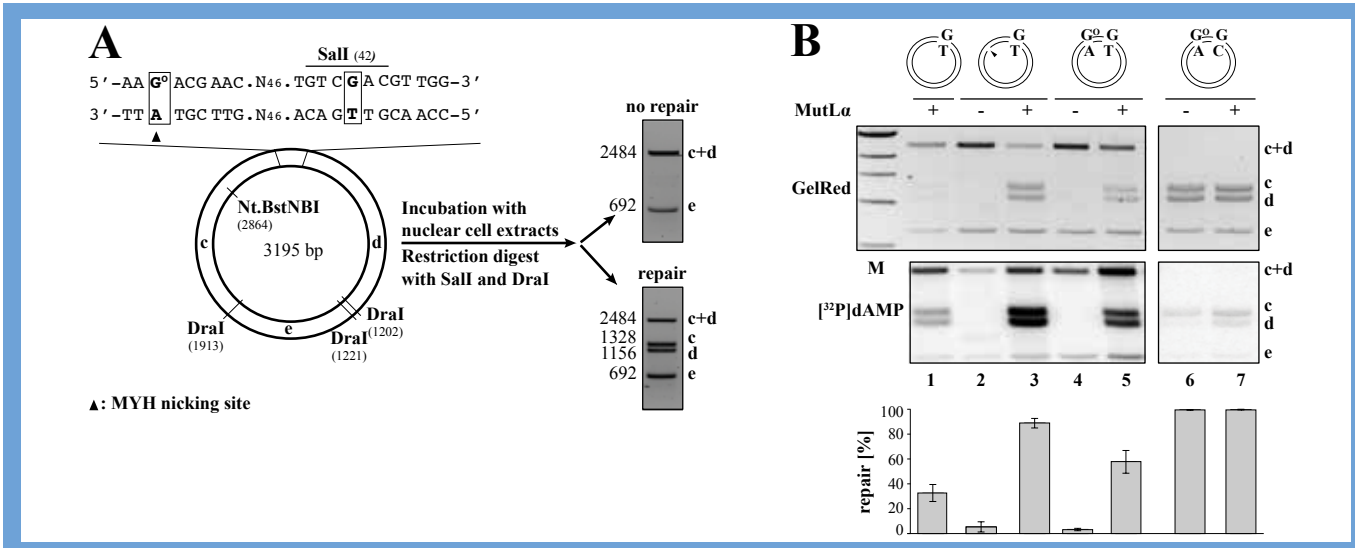

Figure 1. A single G°/A base pair in a DNA heteroduplex can act as an initiation site for MMR in human nuclear cell extracts. (A) Schematic representation of the G°/A-G/T substrate and the in vitro MMR assay. The substrate carries a G°/A mispair 57 nucleotides away from a G/T mismatch, which is located in a SalI recognition site. The presence of the mismatch makes the phagemid refractory to cleavage with the enzyme, such that incubation with SalI and DraI yields fragments c+d (2484 bp) and e (692). (The third, 19 bp long fragment is not detectable on 1% agarose gels stained with GelRed). Presence of a nick in the inner strand, introduced either by Nt.BstNBI or through BER-catalyzed incision of the A strand results in a repair of the G/T mismatch to G/C in human nuclear cell extracts, which regenerates the SalI site. Upon incubation with SalI and DraI, the repaired phagemid gives rise to fragment c (1328 bp), d (1156 bp) and e (692 bp). (B) G°/A mispairs serve as initiation sites for MMR. The MMR assay shows the efficiency of repair of a G/T mismatch in closed-circular G/T (lane 1), nicked G/T (lanes 2-3), closed-circular G°/A-G/T (lanes 4-5) and closed-circular G°/A-G/C (lanes 6-7) substrates that were incubated with nuclear extracts of MutLα-deficient HCT116 cells, supplemented with purified MutLα (+) where indicated. The autoradiograph shows MMR-dependent [^{32}P]dAMP incorporation into the different substrate fragments.

Biochemistry of mismatch repair II

Medini Ghodgaonkar, Katja Kratz and 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. We have also set out to study one of the candidates, the proofreading activity of polymerase-δ. Using a novel approach developed in our laboratory, we have been able to stably replace the endogenous large subunit of pol-δ with a variant that is error-prone, a variant lacking the 3'→ 5' proofreading activity and a third variant affected in both these functions. Phenotypic analysis of these cell lines revealed that all three had substantially elevated mutation frequencies, which implied either that their MMR capacity was saturated, or that MMR failed not address errors that escape the proofreading exonuclease.

We also examined the ability of FAN1, an interactor of the MMR protein MLH1, to compensate for EXO1 deficiency. We could show that FAN1can 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.

Repair of O^6-methylguanine in *Xenopus laevis* egg extracts

Maite Olivera-Harris, Mariela Artola-Borán

The MMR system has been shown to be largely responsible for the cytotoxicity of the mutgenic O^6-methylguanine (^{Me}G), such that MMR-deficient cells are up to 100-fold more resistant to killing by methylating agents of the SN1 type than their MMR-proficient counterparts. This MMR-dependent toxicity was explained by two distinct hypotheses. One posits that MMR attempts to repair ^{Me}G/C and ^{Me}G/T mispairs arising during replication. Because MeG is in the template, MMR addresses the C respectively the T strand. Repair synthesis then regenerates the ^{Me}G/C and ^{Me}G/T mispairs, which trigger further rounds of repir/resynthesis. This futile cycling eventually gives rise to double-strand breaks and cytotoxicity. The second hypothesis states that ^{Me}G/C and ^{Me}G/T mispairs trigger DNA damage response that activates the checkpoint machinery and apoptosis. In vivo experiments could not distinguish between these outcomes, neither could our in vitro MMR system, because the substrate plasmid DNA does not replicate. By generating substrates containing ^{Me}G at a given site, we were able to show that both ^{Me}G/C and ^{Me}G/T mispairs are efficiently addressed by the MMR system in nucleoplasmic extracts of *Xenopus laevis* eggs. Because one round of replication of the ^{Me}G/C substrate failed to trigger DNA damage signalling, our experiments argue in favour of the futile repair hypothesis.

MMR and interstrand cross-link repair

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 (Figure 2). 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 preliminary 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.

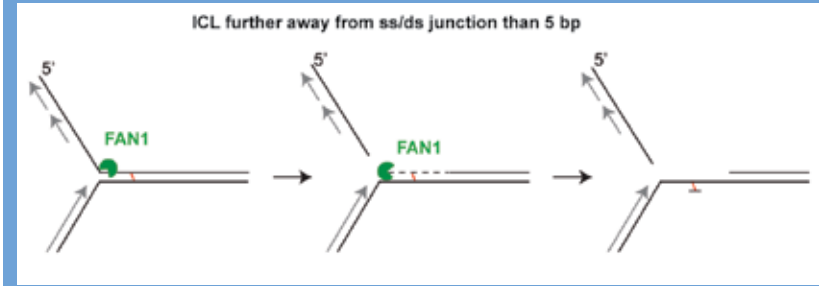

Figure 2.
Putative mechanism of FAN1-dependent ICL unhooking. A replication fork arrested at an ICL that is more than 5 base pairs from the ss/ds boundary would be incised by FAN1 5′ from the ICL, which would release the lagging strand flap. The enzyme would then degrade the nicked strand in a 5′ to 3′ direction to generate a substrate for translesion polymerases and subsequent excision of the crosslink remnant by nucleotide excision repair (NER).

The role of MMR proteins in antibody diversification

Stephanie Bregenhorn, Lia Kallenberger, Mariela Artola-Borán

The generation of our vast antibody repertoire involves three processes: VDJ recombination, somatic hypermutation (SHM) and class switch recombination (CSR). All of these processes irreversibly alter the genome of B cells. Whereas the random recombination of the variable (V), diversity (D) and join (J) regions of the immunoglobulin (Ig) genes takes place in unstimulated B-cells already in the bone marrow, the latter processes are initiated upon antigen stimulation of the cells in germinal centers. SHM/CSR is triggered by activation-induced cytidine deaminase (AID), which is induced in antigen-stimulated B cells and which converts cytosines in certain sequence contexts to uracils. Although uracil processing by base excision repair is generally error-free, in stimulated B cells it gives rise to mutations. Surprisingly, evidence obtained from knock out mouse models and, more recently, also from patients, showed that a subset of these mutations is dependent on MMR. Thus, while MMR is a high-fidelity process, MMR proteins appear to act as mutators during SHM/CSR. Using defined uracil-containing substrates, we could show that base excision repair and MMR compete for the AID-generated U/G mispairs (Figure 3). In instances where the uracil residues are situated in close proximity in opposite DNA strands, the collision of BER and MMR repair tracts can give rise to double-strand breaks, which are believed to be the initiation events in CSR.

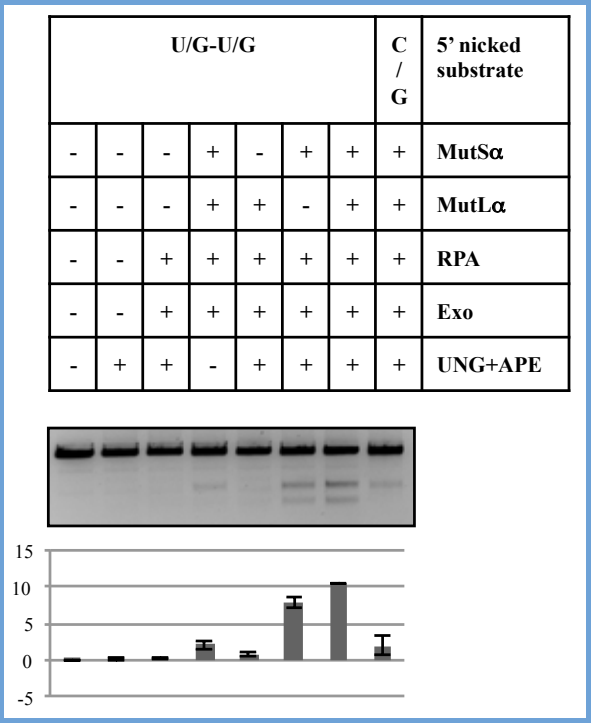

Figure 3.
Reconstitution of the minimal BER/MMR interference system. The minimal mismatch repairosome was assembled from purified recombinant MutSα, MutLα, RPA, EXO1, RFC and PCNA. Uracil processing was mediated by purified recombinant UNG and APE1. Following incubation of the U/G-U/G and nicked U/G-U/G substrates with the indicated protein combinations, the recovered plasmids were subjected to electrophoresis on 0.8% agarose gels. The panel shows an image of a representative gel stained with GelRed. Bar graph shows quantification of three independent experiments. Error bars show mean +/- S.D. (n=3). The figure shows that DSBs are generated only when both BER and MMR proteins are present.

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MASSIMO LOPES

DNA REPLICATION STRESS IN CANCER AND STEM CELLS

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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.

Uncovering the structural determinants of DNA replication stress induced by cancer chemotherapeutics

Marko Vujanovic, Karun Mutreja, Matteo Berti, Jonas Schmid, Ralph Zellweger, Nastassja Terraneo

The 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. We have successfully used our established experimental platform for DNA replication studies of several chemotherapeutic drugs, uncovering surprising alterations of replication fork architecture and refining established models of their action. For example, although replication-induced DSB have long been postulated to mediate the cytotoxicity of topoisomerase inhibitors, we showed that an active control of replication fork progression and architecture – by replication fork slowing and reversal (Figure 1A) - can protect normal and cancer cells from these treatments, when these drugs are used at clinically relevant doses (Ray Chaudhuri et al., *NSMB* 2012). More recently, we showed that replication fork remodeling is a conserved, global response to a full range of genotoxic drugs (Figure 1B), covering practically all different strategies of replication interference typically used in cancer chemotherapy (Zellweger et al., *JCB* 2015). In fact, we have provided solid evidence that these unusual intermediates (reversed forks) accumulate also in the face of endogenous obstacles to replication (Follonier et al., NSMB 2013; Ray Chaudhuri et al., *MCB* 2015), and that they are transient and genome-protective (Neelsen and Lopes, *Nat Rev Mol Cell Biol* 2015). We are currently attempting to elucidate the cellular factors playing a role in the formation and resolution of these structures, as they represent potential targets for cancer chemotherapy. Among these factors, we are actively investigating homologous recombination and *Fanconi anemia* factors, as they were all recently shown to promote genome integrity via replication fork protection, by yet-elusive mechanisms. Furthermore, we are testing the specific contribution of ubiquitin signaling in fine tuning the replication process upon stress. Finally, we are specifically investigating the peculiar transactions that allow replication forks to bypass and repair interstrand crosslinks. These are particularly toxic adducts – induced by byproducts of cell metabolism, as well as several chemotherapeutic drugs – which should represent roadblocks to the replication process, but are in fact mysteriously bypassed at high efficiency during genome replication of human cells.

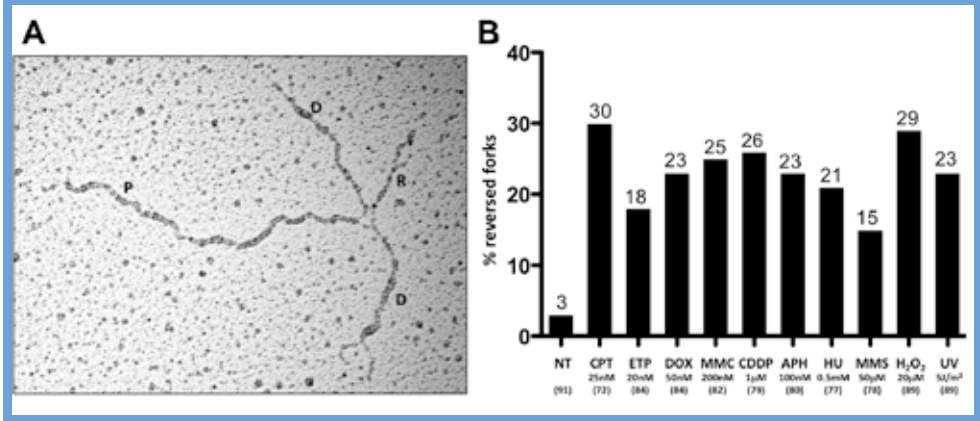

Figure 1.
Mild genotoxic treatments, including cancer chemotherapeutics, result in frequent replication fork reversal.
(A) Electron micrograph of a representative reversed replication fork from U2OS cells treated for 1h with 20nM ETP. P indicates the parental duplex, D indicates daughter duplexes and R indicates the regressed arm.
(B) Frequency of reversed replication forks in U2OS cells either untreated (NT) and upon the indicated treatments. In brackets, the total number of analyzed molecules. Above each column, the percentage of reversed forks is indicated.

DNA replication stress in stem cells

Akshay Ahuja, Kurt Jacobs

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. *Nat. 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 of these cells (Figure 2B; Ahuja et al. *Nat. Comms* 2016). We are now expanding these studies, by actively investigating whether similar surprising phenomena can be observed in early embryogenesis of other organisms. Furthermore - in light of the recently proposed links between adult stem cell proliferation, DNA damage and cancer - we are studying whether activation of quiescent stem cells leads to detectable replication stress phenotypes.

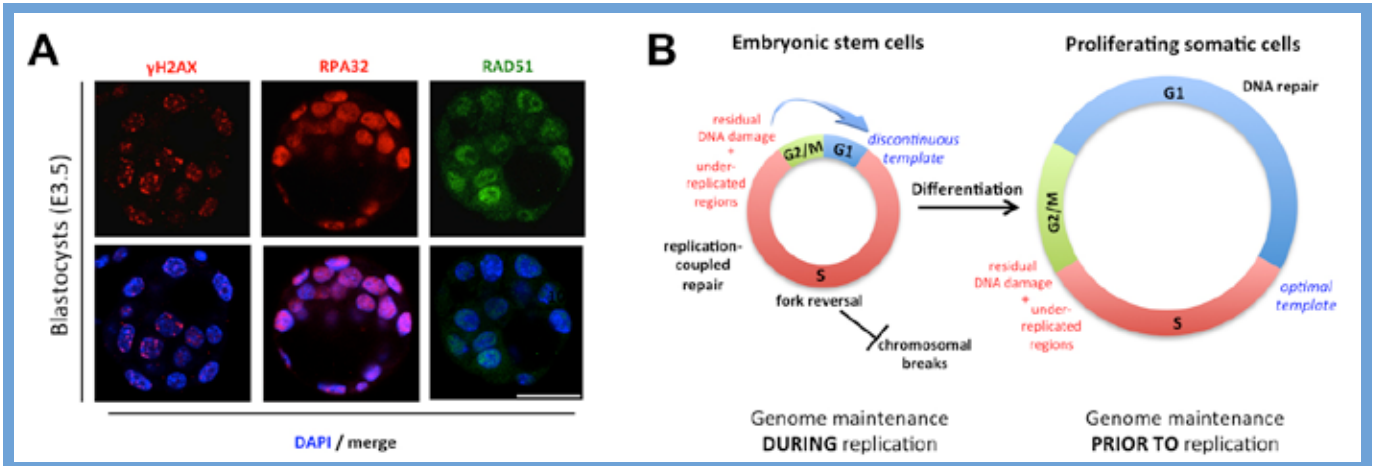

Figure 2.
Replication stress markers in embryonic stem cells and their alternative, replication-coupled strategy for genome maintenance.
(A) Immunofluorescence staining for the DNA damage-marker γH2AX, and for chromatin binding of the ssDNA-binding proteins RPA32 and RAD51 in E3.5 blastocysts. The results 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, assemble 53BP1 nuclear bodies 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.

Structural insights into DNA replication stress in cancer onset

Sofija Mijic, Katharina Zwicky

DNA damage response is a critical anti-tumour barrier that prevents the proliferation of cells with potentially hazardous genetic alterations. It acts early in tumorigenesis and its activation was observed already in pre-cancerous lesions of various organs. The activation of the DNA damage checkpoint in these lesions was ascribed to oncogene-induced deregulation of DNA synthesis, or "replication stress". Although the indirect consequences of replication stress, i.e. cell cycle arrest and senescence, have been elucidated to some extent, our understanding of the underlying molecular events is extremely vague. This is mainly due to the lack of information on the *in vivo* DNA structures generated under such conditions.

The replication stress phenotype can be reproduced in cell culture by overexpression of various oncogenes influencing DNA replication, or by inactivation of crucial cell cycle controls, leading to over-replication of the genome. We have exploited these systems to identify oncogene-associated defects in DNA replication. Overexpression of different oncogenes (e.g. CyclinE and CDC25A) has a substantial effect on bulk DNA synthesis (Figure 3A-B) and leads to a marked slow-down of individual replication forks, measured by FACS analysis and DNA fiber labelling, respectively (Neelsen et al., *JCB* 2013). Furthermore, electron microscopic analysis (Neelsen et al., *MiMB* 2014) reveals the accumulation of aberrant replication intermediates. When oncogene activation is coupled to inactivation of cell cycle checkpoints, unscheduled processing of these unusual intermediates leads to massive DNA breakage and full DDR activation, associated with premature mitotic entry. Using a similar set of approaches we have also characterized the molecular consequences of "re-replication" (Figure 3C; Neelsen et al., *G&D* 2013), a deregulation of replication initiation program that is frequently associated with tumorigenesis. We are currently extending our studies to a broad spectrum of oncogenes, to possibly extract common molecular mechanisms underlying tumorigenesis from its earliest stages. Furthermore, we have recently started investigating replication-transcription interference, which is supposedly associated with the accumulation of toxic DNA-RNA hybrids (R-loops) and was recently shown to underlie the tumorigenic potential of several oncogenes.

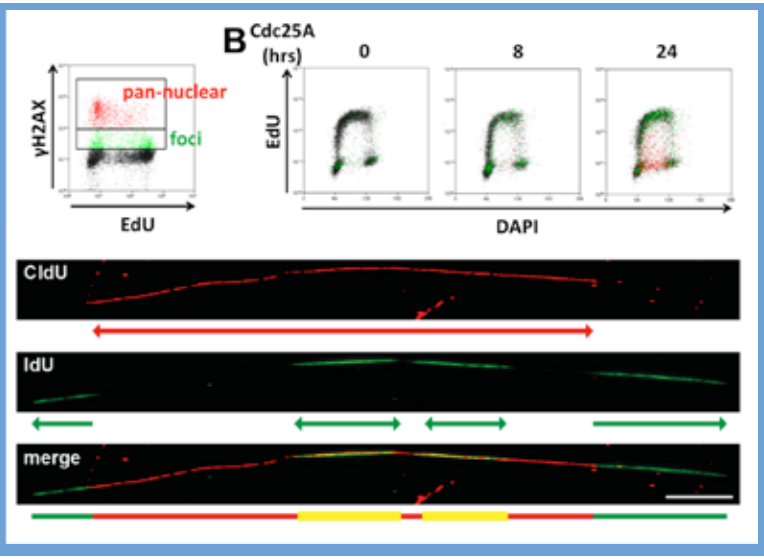

Figure 3.
Flow cytometric and single molecule analysis of DNA replication stress by oncogene activation or deregulated initiation.
(A) FACS-based distinction of γH2AX patterns after Cdc25A induction. Red and green signals indicate cells with pan-nuclear γH2AX and γH2AX foci respectively, indicative of active DNA damage response.
(B) FACS analysis after Cdc25A induction shows accumulation of cells with γH2AX foci and pan-nuclear staining. Pan-nuclear γH2AX is associated with replicative arrest.
(C) Representative DNA tract labelled with CldU (red) for 2 h and IdU (green) for 30 min and spread by DNA fiber assay to identify re-replication events. This molecule shows two "re-replication" events (yellow tracts) in close proximity.

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Aberrant activation of the Wnt signaling pathway that controls epithelial cell division and migration, and defects in DNA mismatch repair that is responsible for the correction of DNA replication errors, have been implicated, among other molecular alterations, in the etiogenesis of colon cancer. Our goal is to understand how the malfunction of these molecular pathways leads to cellular transformation and, in the long run, to translate this knowledge into a direct benefit for individuals afflicted with this disease.

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, these 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, transcriptomics, proteomics, 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.

A comprehensive look at transcription factor gene expression changes in colorectal adenomas

Vonlanthen J, Okoniewski MJ, Menigatti M, Cattaneo E, Pellegrini-Ochsner D, Haider R, Jiricny J, Staiano T, Buffoli F, Marra G

Background: Biological processes are controlled by transcription networks. Expression changes of transcription factor (TF) genes in precancerous lesions are therefore crucial events in tumorigenesis. Our aim was to obtain a comprehensive picture of these changes in colorectal adenomas.

Methods: Using a 3-pronged selection procedure, we analyzed transcriptomic data on 34 human tissue samples (17 adenomas and paired samples of normal mucosa, all collected with ethics committee approval and written, informed patient consent) to identify TFs with highly significant tumor-associated gene expression changes whose potential roles in colorectal tumorigenesis have been underresearched. Microarray data were subjected to stringent statistical analysis of TF expression in tumor vs. normal tissues, MetaCore-mediated identification of TF networks displaying enrichment for genes that were differentially expressed in tumors, and a novel quantitative analysis of the publications examining the TF genes' roles in colorectal tumorigenesis.

Results: The 261 TF genes identified with this procedure included DACH1, which plays essential roles in the proper proliferation and differentiation of retinal and leg precursor cell populations in Drosophila melanogaster. Itspossible roles in colorectal tumorigenesis are completely unknown, but it was found to be markedly overexpressed (mRNA and protein) in all colorectal adenomas and in most colorectal carcinomas. However, DACH1 expression was absent in some carcinomas, most of which were DNA mismatch-repair deficient. When networks were built using the set of TF genes identified by all three selection procedures, as well as the entire set of transcriptomic changes in adenomas, five hub genes (TGFB1, BIRC5, MYB, NR3C1, and TERT) where identified as putatively crucial components of the adenomatous transformation process.

Conclusion: The transcription-regulating network of colorectal adenomas (compared with that of normal colorectal mucosa) is characterized by significantly altered expression of over 250 TF genes, many of which have never been investigated in relation to colorectal tumorigenesis.

Keywords: Transcription factors; Gene expression; Colorectal adenomas; DACH1.

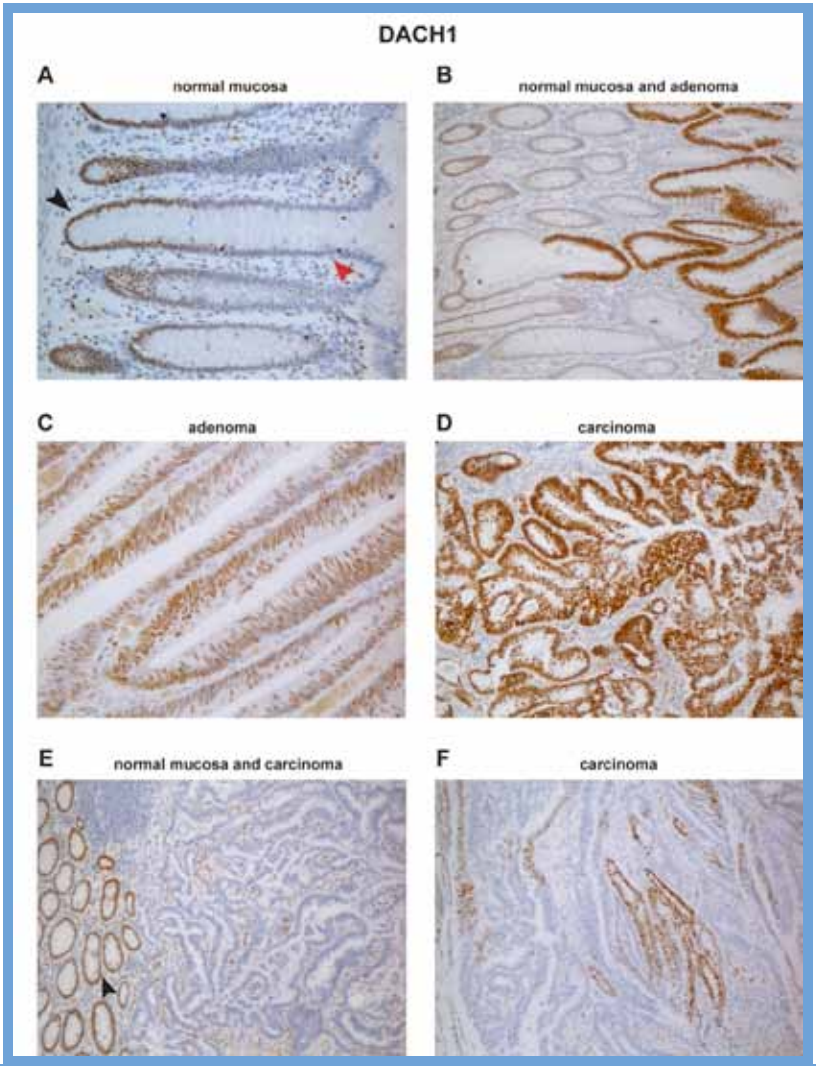

Figure 1.
Immunohistochemical staining for DACH1 protein in normal and neoplastic colon. (A) In normal mucosa, DACH1 expression is present in the nuclei of proliferating cells in the lower portion of the epithelial crypts (black arrowhead) and completely absent in the differentiated cells in the upper crypts (red arrowhead). (B) High-level DACH1 expression is seen in rapidly proliferating cells of adenomatous glands taking over normal crypts. Abundant expression is also seen in most cells of a colorectal adenoma (C) and a colorectal carcinoma (D). In another colorectal cancer (E), DACH1 expression is absent in neoplastic glands, although proliferating cells in the normal mucosa and in the tumoral stroma are positive. (F) A third colorectal cancer with patchy staining for DACH1. From BMC Cancer. 2014 Jan 29;14:46

Sorbitol dehydrogenase overexpression and other aspects of dysregulated protein expression in human precancerous colorectal neoplasms: a quantitative proteomics study

Uzozie A, Nanni P, Staiano T, Grossmann J, Barkow-Oesterreicher S, Shay JW, Tiwari A, Buffoli F, Laczko E, Marra G

Colorectal adenomas are cancer precursor lesions of the large bowel. A multitude of genomic and epigenomic changes have been documented in these preinvasive lesions, but their impact on the protein effectors of biological function has not been comprehensively explored. Using shotgun quantitative MS, we exhaustively investigated the proteome of 30 colorectal adenomas and paired samples of normal mucosa. Total protein extracts were prepared from these tissues (prospectively collected during colonoscopy) and from normal (HCEC) and cancerous (SW480, SW620, CACO2, HT29, CX1) colon epithelial cell lines. Peptides were labeled with isobaric tags (iTRAQ 8-plex), separated by OFFGEL electrophoresis, and analyzed by LC-coupled tandem MS. Non-redundant protein families (4325 in tissues, 2017 in cell lines) were identified and quantified. Principal component analysis of the results clearly distinguished adenomas from normal mucosal samples, and cancer cell lines from HCEC cells. Two hundred twelve proteins displayed significant adenoma-related expression changes (q-value < 0.02, mean fold change vs. normal mucosa +/-1.4), which correlated (r=0.74) with similar changes previously identified by our group at the transcriptome level. Fifty-one (~25%) proteins displayed directionally similar expression changes in colorectal cancer cells (vs. HCEC cells) and were therefore attributed to the epithelial component of adenomas. Although benign, adenomas already exhibited cancer-associated proteomic changes: 69 (91%) of the 76 protein upregulations identified in these lesions have already been reported in cancers. One of the most striking changes involved sorbitol dehydrogenase (SORD), a key enzyme in the polyol pathway. Validation studies revealed dramatically increased SORD concentrations and activity in adenomas and cancer cell lines, along with important changes in the expression of other enzymes in the same (AKR1B1) and related (KHK) pathways. Dysregulated polyol metabolism may represent a novel facet of the metabolome remodeling associated with tumorigenesis.

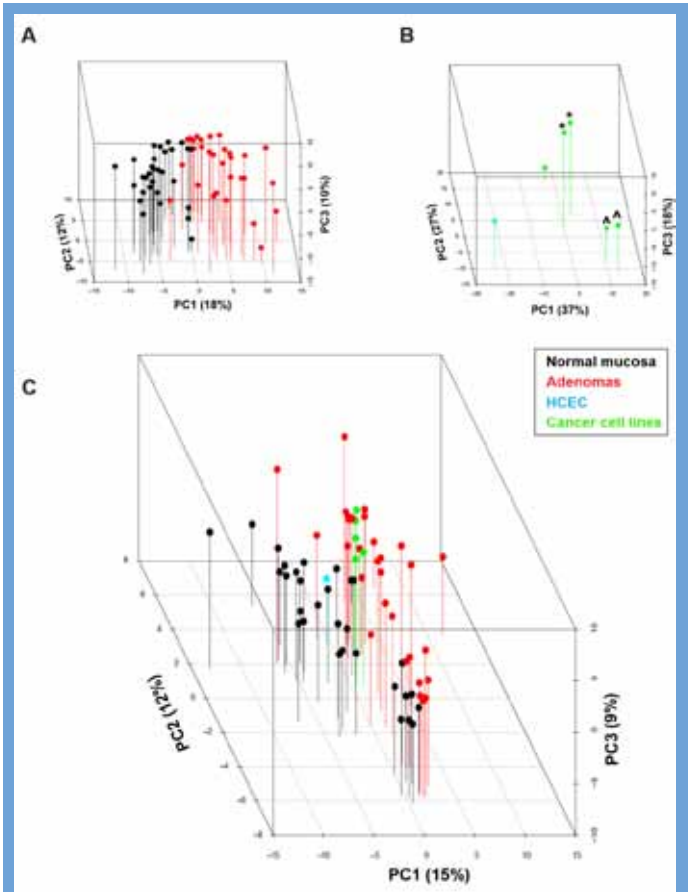

Figure 2.
Three-dimensional principal component analysis score plot of log2 protein expression intensity values for (A) tissues (normal mucosa, black; adenomas, red), (B) cell lines (HCEC, cyan; colon cancer cell lines, green), and (C) both.
From Mol Cell Proteomics. 2014 May;13(5):1198-218.

Early insights into the function of KIAA1199, a markedly overexpressed protein in human colorectal tumors

Tiwari A, Schneider M, Fiorin A, Haider R, Okoniewski M J, Roschitzki B, Uzozie A, Menigatti M, Jiricny J, Marra G

We previously reported that the expression of *KIAA1199* in human colorectal tumors (benign and malignant) is markedly higher than that in the normal colonic mucosa. In this study, we investigated the functions of the protein encoded by this gene, which are thus far unknown. Immunostaining studies were used to reveal its subcellular localization, and proteomic and gene expression experiments were conducted to identify proteins that might interact with KIAA1199 and molecular pathways in which it might play roles. Using colon cancer cell lines, we showed that both endogenous and ectopically expressed KIAA1199 is secreted into the extracellular environment. In the cells, it was found mainly in the perinuclear space (probably the ER) and cell membrane. Both cellular compartments were also over-represented in lists of proteins identified by mass spectrometry as putative KIAA1199 interactors and/or proteins encoded by genes whose transcription was significantly changed by KIAA1199 expression. These proteomic and transcriptomic datasets concordantly link KIAA1199 to several genes/proteins and molecular pathways, including ER processes like protein binding, transport, and folding; and Ca^{2+}, G-protein, ephrin, and Wnt signaling. Immunoprecipitation experiments confirmed KIAA1199's interaction with the cell-membrane receptor ephrin A2 and with the ER receptor ITPR3, a key player in Ca^{2+} signaling. By modulating Ca^{2+} signaling, KIAA1199 could affect different branches of the Wnt network. Our findings suggest it may negatively regulate the Wnt / CTNNB1 signaling, and its expression is associated with decreased cell proliferation and invasiveness.

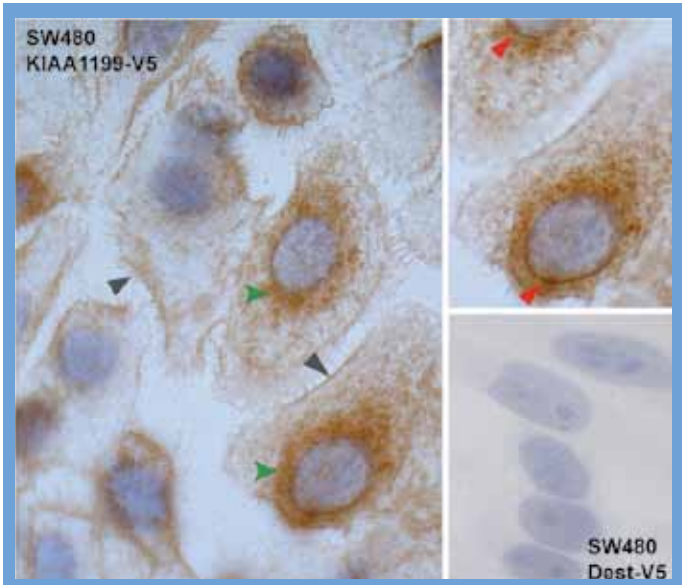

Figure 3.
Immunocytochemical staining of SW480 colon cancer cells with or without expression of KIAA1199. In SW 480 KIAA1199-V5 cells, KIAA1199 is localized in the cell membrane (gray arrowheads) and perinuclear space (green arrowheads). Upper right inset: Fine focusing clearly reveals staining of the nuclear membrane (red arrowheads). Lower right inset: Negative control (SW480 Dest-V5 immunostained with V5-tag-specific antibody).
From Plos One 2013, 8(7): e69473.

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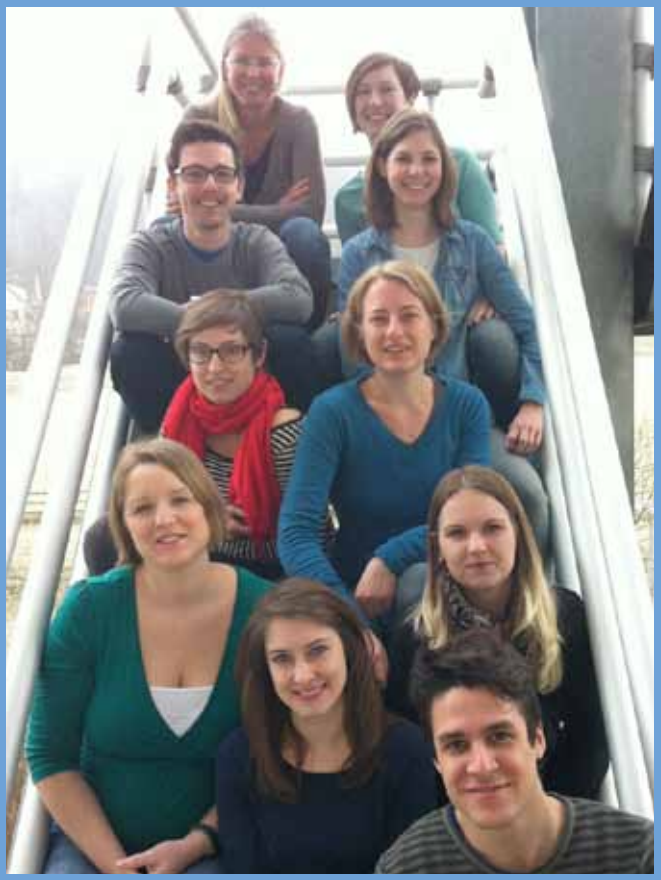
Swiss National Science Foundation, Swiss Cancer League , Univeristy Research Priority Program

ANNE MÜLLER

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.

Helicobacter pylori-induced DNA strand breaks are introduced by nucleotide excision repair endonucleases and promote NF-κB target gene expression

Mara Hartung, Dorothea Gruber

Multiple species of pathogenic bacteria have been shown in recent years to damage the nuclear DNA of their host cells, often resulting in particularly hazardous DNA double strand breaks (DSBs). The carcinogenic bacterial pathogen *H. pylori*, which causes chronic gastritis and is the most important risk factor for the development of peptic ulcer disease and gastric cancer, also exhibits genotoxic activity. Exposure of gastric epithelial cells to *H. pylori* induces DNA fragmentation in a contact-dependent manner that is detectable by pulsed field gel electrophoresis (PFGE) and can be visualized by high resolution microscopy of metaphase chromosomes; the DSB repair factor p53 binding protein 1 (53BP1) and others are recruited to the sites of DSBs, and, together with the phosphorylation of histone H2A variant X (γH2AX), indicate the initiation of DSB repair by the host cell repair machinery. We have begun to investigate the mechanistic basis of *H. pylori*-induced DNA DSBs and to elucidate key host and bacterial factors contributing to DSB induction (summarized in Figure 1). By screening an H. pylori transposon library for mutants lacking the ability to induce host cell DNA DSBs, we have identified the cag pathogenicity island (*cag*PAI)-encoded T4SS as being critically involved in *H. pylori*-induced DNA damage. We have further found active transcription and the T4SS/β1 integrin-induced activation of nuclear factor kappa B (NF-κB) to be important prerequisites of DSB induction and have identified the nucleotide excision repair (NER) endonucleases XPF and XPG as critical enzymes introducing the strand breaks. XPF/XPG-mediated DSBs serve to amplify NF-κB target gene expression and to promote host cell survival. Ongoing projects are designed to further elucidate the mechanistic basis and functional consequences of *H. pylori* induced DNA DSBs for the host/*H. pylori* interaction and gastric carcinogenesis. See Hartung et al., Cell Reports, 2015.

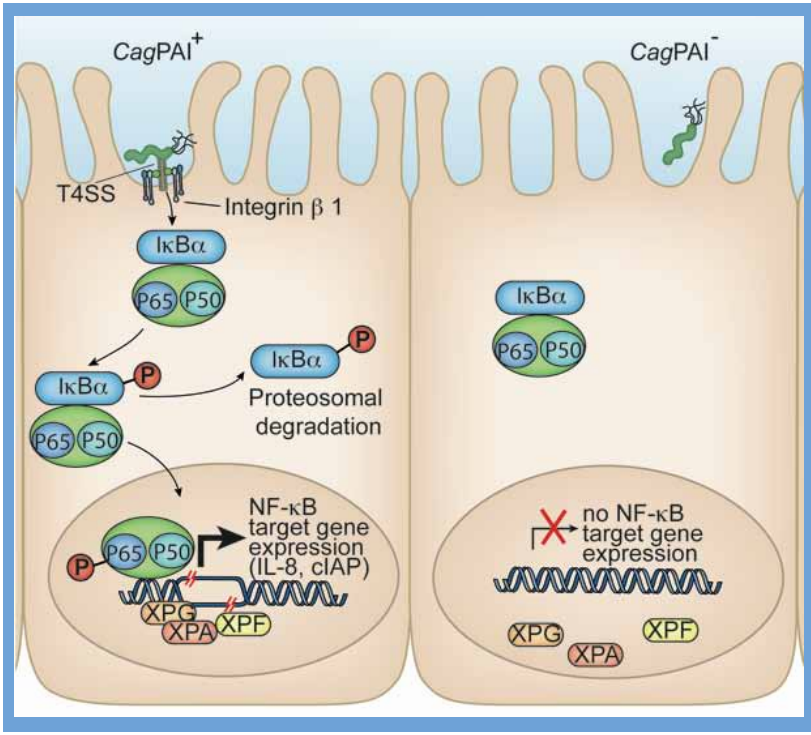

Figure 1.
The human bacterial pathogen *Helicobacter pylori* exhibits genotoxic properties that potentially promote gastric carcinogenesis. *H. pylori* introduces DNA double strand breaks (DSBs) in epithelial cells that trigger host cell DNA repair efforts. *H. pylori*-induced DSBs are repaired via error-prone, potentially mutagenic non-homologous end joining. The *H. pylori* type IV secretion system is required for DSB generation. The DSBs are introduced by the nucleotide excision repair endonucleases XPF and XPG and promote NF-κB target gene transactivation.

Molecular pathogenesis of diffuse large B-cell lymphoma

DUSP4 deficiency due to promoter hypermethylation drives JNK signaling and tumor cell survival in diffuse large B-cell lymphoma

Corina A. Schmid

The epigenetic dysregulation of tumor suppressor genes is an important driver of human carcinogenesis. We have combined genome-wide DNA methylation analyses and gene expression profiling after pharmacological DNA demethylation with functional screening to identify novel tumor suppressors in diffuse large B-cell lymphoma (DLBCL). We find that a CpG island in the promoter of the dual-specificity phosphatase DUSP4 is aberrantly methylated in nodal and extranodal DLBCL, irrespective of ABC or GC subtype, resulting in loss of DUSP4 expression in 75% of >200 examined cases. The *DUSP4* genomic locus is further deleted in up to 13% of aggressive B-cell lymphomas and the lack of DUSP4 is a negative prognostic factor in three independent cohorts of DLBCL patients (Figure 2). Ectopic expression of wild-type DUSP4, but not of a phosphatase-deficient mutant, dephosphorylates c-JUN N-terminal kinase (JNK) and induces apoptosis in DLBCL cells. Pharmacological or dominant-negative JNK inhibition restricts DLBCL survival *in vitro* and *in vivo,* and synergizes strongly with the Bruton's tyrosine kinase inhibitor ibrutinib. Our results indicate that DLBCL cells depend on JNK signaling for survival. This finding provides a mechanistic basis for the clinical development of JNK inhibitors in DLBCL, ideally in synthetic lethal combinations with inhibitors of chronic active B-cell receptor signaling. Further reading: Schmid et al., Journal of Experimental Medicine, 2015.

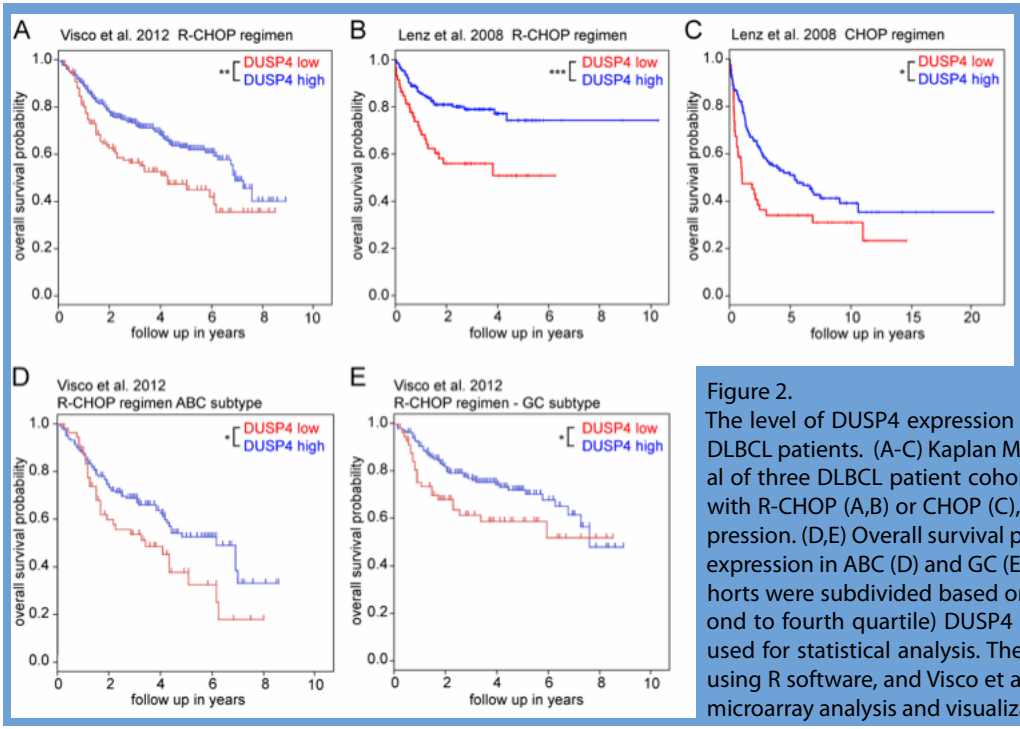

Figure 2.
The level of DUSP4 expression correlates with overall survival of DLBCL patients. (A-C) Kaplan Meier curves showing overall survival of three DLBCL patient cohorts (GSE31312, GSE10846) treated with R-CHOP (A,B) or CHOP (C), as a function of DUSP4 mRNA expression. (D,E) Overall survival probability based on DUSP4 mRNA expression in ABC (D) and GC (E) subsets of DLBCL patients. All cohorts were subdivided based on low (first quartile) and high (second to fourth quartile) DUSP4 expression. The log-rank test was used for statistical analysis. The Lenz et al. dataset was analyzed using R software, and Visco et al. data was analyzed using the R2: microarray analysis and visualization platform (http://r2.amc.nl).

The hematopoietic oncoprotein FOXP1 promotes tumor cell survival in diffuse large B-cell lymphoma by repressing S1PR2 signaling

Michael Flori

Aberrant expression of the oncogenic transcription factor FOXP1 is a common feature of diffuse large B-cell lymphoma (DLBCL). We have combined chromatin immunoprecipitation and gene expression profiling after FOXP1 depletion with functional screening to identify targets of FOXP1 contributing to tumor cell survival. We find that the sphingosine-1-phosphate receptor 2 (S1PR2) is repressed by FOXP1 in activated B-cell (ABC) and germinal center B-cell (GCB) DLBCL cell lines with aberrantly high FOXP1 levels; S1PR2 expression is further inversely correlated with FOXP1 expression in three patient cohorts. Ectopic expression of wild type S1PR2, but not of a point mutant incapable of activating downstream signaling pathways, induces apoptosis in DLBCL cells and restricts tumor growth in subcutaneous and orthotopic models of the disease. The pro-apoptotic effects of S1PR2 are phenocopied by ectopic expression of the small G-protein Gα13, but are independent of AKT signaling. We further show that low S1PR2 expression is a strong negative prognosticator of patient survival, alone and especially in combination with high FOXP1 expression. The *S1PR2* locus has previously been demonstrated to be recurrently mutated in GCB DLBCL; the transcriptional silencing of *S1PR2* by FOXP1 represents an alternative mechanism leading to inactivation of this important hematopoietic tumor suppressor (see schematic in Figure 3 for details). Further reading: Flori et al., Blood 2016.

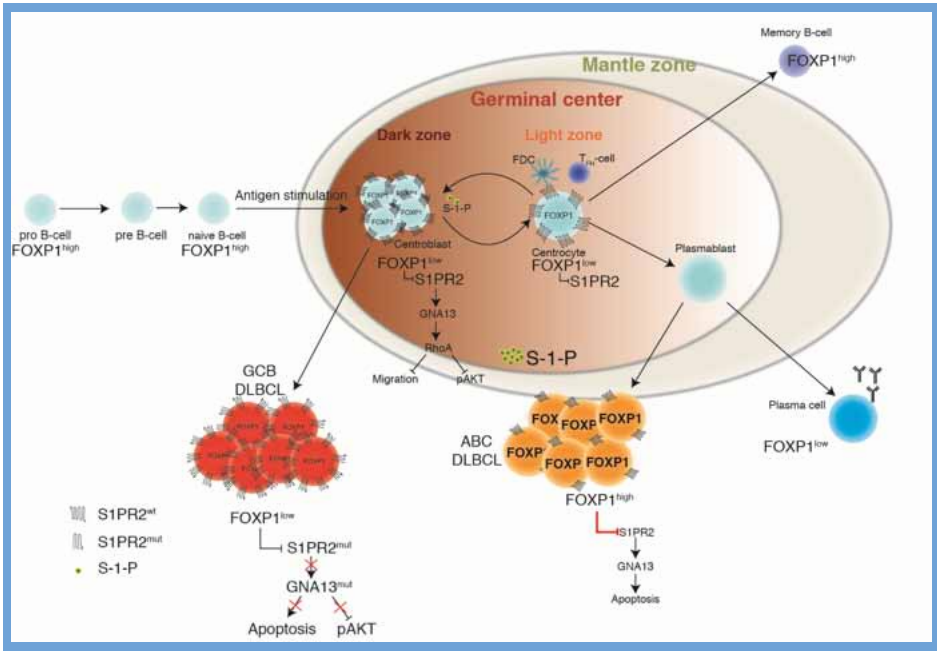

Figure 3. FOXP1 represses S1PR2 expression and downstream signaling pathways to promote cell survival in DLBCL. Scheme showing the FOXP1/S1PR2-regulated signaling pathways in normal and malignant B-cells. Under physiological conditions, a gradient of S1P with highest concentrations outside the germinal center (GC) and at the GC boundary inhibits migration and survival of FOXP1^{lo} S1PR2^{hi} GC B cells through S1PR2-mediated inhibition of RhoA and AKT signaling. In FOXP1-overexpressing (generally ABC-type) DLBCL, the transcriptional repression of S1PR2 promotes cell survival. In contrast, in FOXP1^{lo} GCB DLBCL, the *S1PR2* genomic locus is often mutated in coding as well as non-coding regions, leading to functional inactivation of S1PR2. Ectopic restoration of S1PR2 levels in ABC-type as well as GCB-type DLBCL cell lines induces apoptosis in a Gα13-dependent manner.

Helicobacter urease activates the TLR2/NLRP3/IL-18 axis to protect against asthma

Katrin Koch, Sabine Urban, Andreas Kyburz

Inflammasome activation and the caspase-1-dependent processing and secretion of IL-1β and IL-18 are critical events at the interface of the bacterial pathogen *Helicobacter pylori* with its host. Whereas IL-1β promotes Th1 and Th17 responses and gastric immunopathology, IL-18 is required for Treg differentiation, *H. pylori* persistence and the protection against allergic asthma that is a hallmark of *H. pylori*-infected mice and humans. Here we show that inflammasome activation in DCs requires the cytoplasmic sensor NLRP3 as well as TLR2 signaling induced by *H. pylori*. Screening of an *H. pylori* transposon mutant library revealed distinct roles for the bacterial LPS in proIL-1β expression, and for the urease B subunit in NLRP3 inflammasome licensing (see schematic in Figure 4). UreB activates the TLR2-dependent expression of NLRP3, which represents a rate-limiting step in NLRP3 inflammasome assembly. *UreB* gene deletion mutants are defective for caspase-1 activation in murine bone-marrow-derived and splenic DCs as well as human blood-derived DCs. Despite colonizing the murine stomach, ureB mutants fail to induce IL-1β and IL-18 secretion and to promote Treg responses. Unlike wild type *H. pylori, ureB* mutants are incapable of conferring protection against allergen-induced asthma, indicating a critical contribution of the TLR2/NLRP3/caspase-1/IL-18 axis to *H. pylori*-specific immune regulation. Further reading: Koch et al. Journal of Clinical Investigation, 2015.

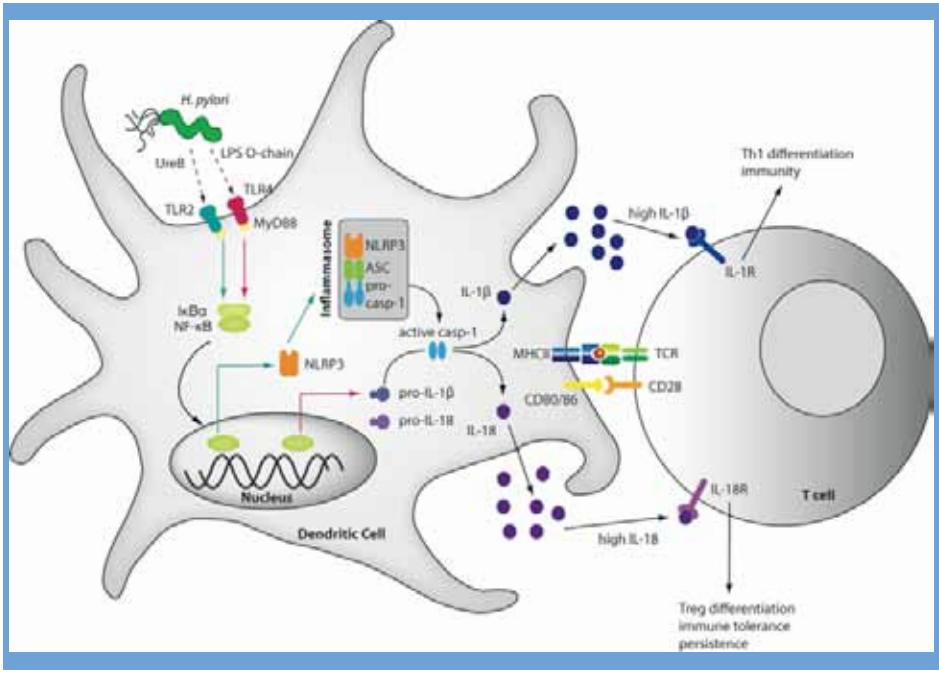

Figure 4. Scheme of *H. pylori*-induced inflammasome activation, cytokine processing and downstream effects. *H. pylori* LPS and the urease B subunit (UreB) promote NLRP3 inflammasome and caspase-1 activation as well as IL-1β and IL-18 processing and secretion. *H. pylori* LPS activates IL-1β expression *via* TLR4, MyD88 and NF-κB (indicated by red arrows), whereas UreB signals *via* TLR2, MyD88 and NF-κB to activate NLRP3 transcription (green arrows). The assembly of NLRP3, ASC and pro-caspase-1 is triggered through an as yet unknown mechanism, leading to caspase-1 activation and to the processing of pro-IL-1β and pro-IL-18. The mature cytokines are released, bind to their receptors on naive T-cells and promote Th1 differentiation and *H. pylori* control in the case of IL-1β, and Treg differentiation, immune tolerance and persistence in the case of IL-18. The pro-form of IL-18 is constitutively expressed in DCs.

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LORENZA PENENGO

UBIQUITIN CONTROL OF GENOME STABILITY

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Our research focuses on the role of ubiquitination and ubiquitin-like modifications in the regulation of different aspects of the DNA metabolism. 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.

Eukaryotic cells have developed efficient ways to modulate the properties of proteins, in order to rapidly respond to variations of external conditions and to face potentially dangerous external events. Among them is the reversible, covalent attachment of modifying groups including small entities such as phosphate or acetyl group, but also entire proteins, such as members of the ubiquitin (Ub) family. Ub is a 76 amino acid polypeptide that has been found appended to lysine residues of many proteins. A cascade of enzymes is required for the ubiquitination reaction. After the first Ub monomer, additional Ub molecules can be attached to the target protein through any of the eight amino groups in the first molecule - the N-terminus (M1), K6, K11, K27, K29, K33, K48, and K63 - to form polyubiquitin chains. These different linkages increase the complexity of the Ub system, giving rise to Ub chains with distinct topology and thus providing structural flexibility that results in a multitude of functional outcomes. Although the roles of K48 and K63 polyUb in protein degradation and cellular signalling are well characterized, the functions of the remaining six non-canonical Ub chain types are still largely obscure.

Ubiquitination is essential for almost all cellular processes, including the maintenance of genome stability. Indeed, formation of DNA double strand breaks (DSBs) induced by genotoxic stress triggers a cascade of phosphorylation and ubiquitination events - the DDR - allowing the relaxation of chromatin structure and the recruitment of important downstream effectors, such as 53BP1 and BRCA1, which regulate DNA repair. Inhibition of these Ub-mediated events has detrimental consequences for the cell.

A crucial role in this pathway is played by the Ub ligase RNF168, which ubiquitinates histones H2A and H2A.X and allows the activation of DDR (Pinato et al, BMC Mol Biol 2009; Pinato et al, MCB 2011). Inactivation of RNF168, either by point mutation or by depletion, completely blocks the pathway, increasing sensitivity of cells to genotoxic agents (Figure 1). The relevance of ubiquitination, and in particular of RNF168, in the maintenance of genome stability has been directly demonstrated by the identification of two mutations in RNF168 gene as responsible of the RIDDLE (radiosensitivity, immunodeficiency, learning difficulties and dysmorphic features) syndrome, a recently described disorder characterised by cancer predisposition. These findings clearly indicate that chromatin ubiquitination, and RNF168 itself, play a pivotal role in the maintenance of genome stability and that its alteration may predispose to cancer development. Similarly, they identify the ubiquitination pathway as a potential therapeutic target.

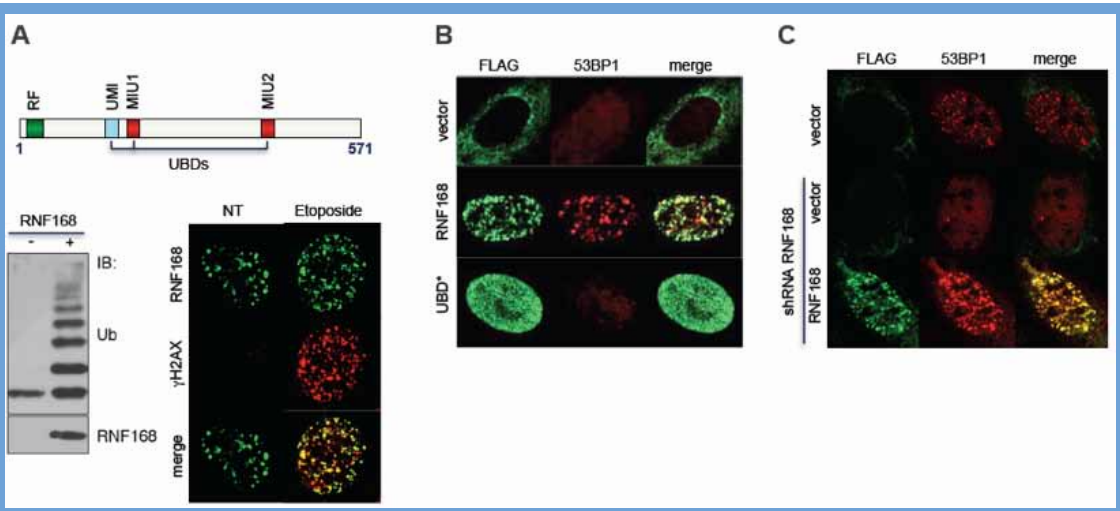


Figure 1. (A) Schematic representation of RNF168: RF, RING finger domain; UMI, MIU1 and MIU2 are 3 Ub binding domains, UBDs. Ectopic expression of RNF168 induces extensive chromatin ubiquitination, as indicated by Ub immunoblotting (IB). Immunofluorescence analysis shows that RNF168 is recruited to the sites of DNA damage - called DDR foci – as revealed by co-localization with specific markers (phospho-H2A.X or γH2AX). (B and C) RNF168 is strictly required for proper activation of DDR: both point mutations addressing its Ub binding ability (UBDs*) and knock-down of the protein by shRNA targeting RNF168 result in the block of 53BP1 recruitment to DDR foci.

RNF168 triggers a specific Ub-based mark on chromatin to signal DNA damage

For a long time it has been assumed that when cells are exposed to genotoxic agents, chromatin is modified by a specific type of modification, i.e. K63-linked ubiquitination, targeting H2A family of histones at a unique site at the C-terminal tail of histone H2A (on residue K119). Now, we added new mechanistic insights into Ub-based remodelling of chromatin, by demonstrating the following:

UbK13/K15: a novel ubiquitination site on histone H2As induced by genotoxic stress. We discovered that DNA lesions, instead of elongating Ub chain on K119, generate a novel Ub signature on the N-terminal tail of histone H2A and H2A.X (UbK13/K15) to transmit a specific signal to downstream effectors (Figure 2). This novel Ub mark, generated by RNF168, is qualitatively different from the canonical UbK119 and warns the cell to activate DNA repair (Gatti et al, Cell Cycle 2012).

RNF168 promotes K27-linked non-canonical ubiquitination to signal DNA damage. Using a combination of molecular biology, biochemistry, specialized mass spectrometric analysis and a cell-based assay, we recently obtained novel results showing that RNF168 activates the DDR by using K27- and not K63-linked ubiquitination (Figure 3). We demonstrated that K27 ubiquitination is the major Ub-based modification marking chromatin upon DNA damage and that it is strictly required for the proper activation of the DDR (Gatti et al, Cell Reports 2015).

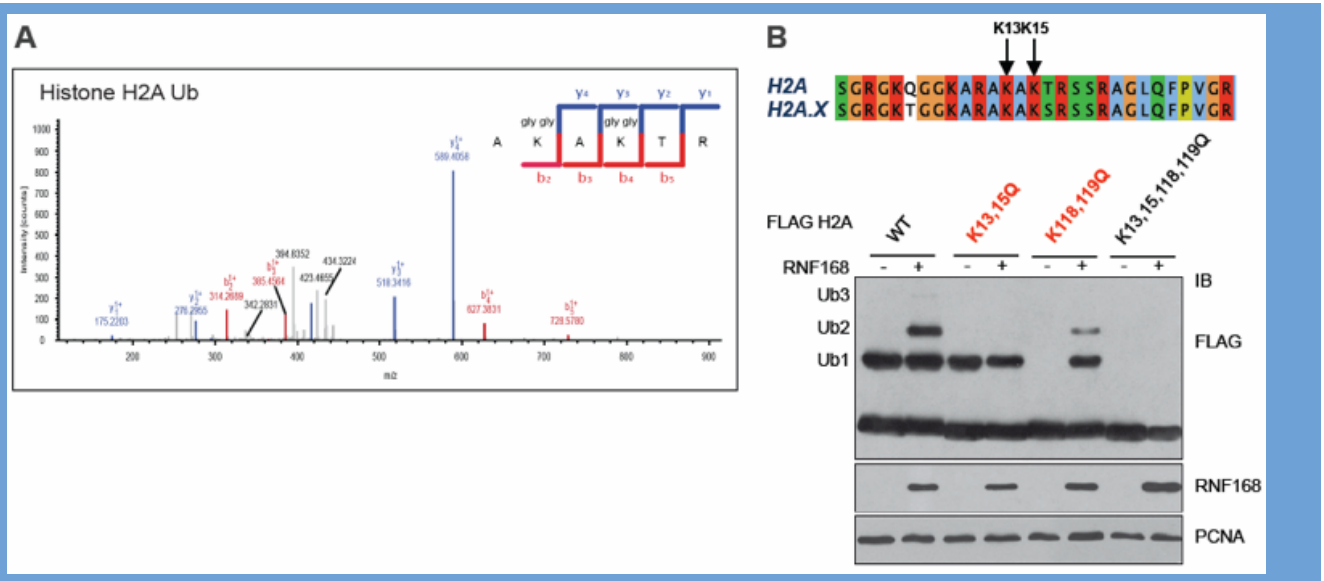
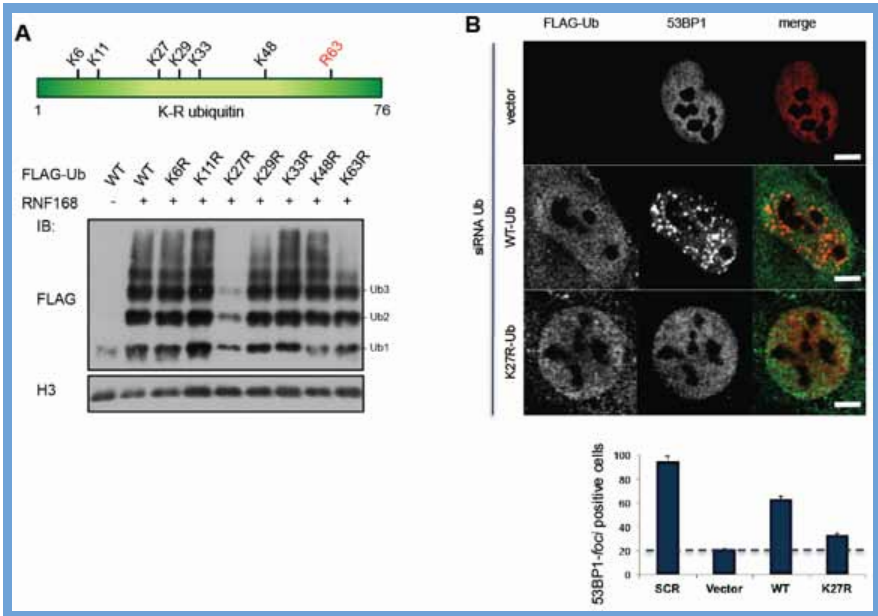


Figure 2. (A) Mass spectrometric analysis of chromatin extracts derived from cells expressing RNF168 revealed a novel ubiquitination site on histone H2As, formed by K13/K15 residues. (B) This site is embedded in a highly conserved sequence. Mutation of the K13/K15 site maintains monoubiquitination of H2A (revealed by FLAG IB) but prevents further ubiquitination (Ub2, Ub3) induced by RNF168. In contrast, a mutant affecting the canonical K118/K119 site is unable to form monoUb H2A in the absence of RNF168, but it ubiquitination is fully restored upon RNF168 expression.



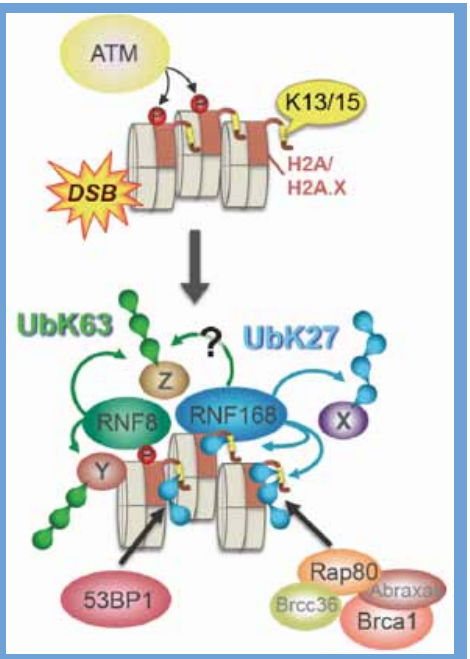


Figure 3. (A) Schematic example of the Ub K-R mutants used in the experiment. Chromatin ubiquitination is considerably impaired in cells expressing the K27R mutant of Ub, but less so in cells expressing the other mutants. (B) Knock-down of Ub (siRNA Ub) completely blocks the recruitment of 53BP1 to the sites of lesions (see vector). DDR foci are restored by the expression of a siRNA-resistant form of Ub (WT-Ub), but not by the K27R mutant, clearly indicating a pivotal role of the K27 linkage in this process.

WORKING MODEL

Formation of DSBs by genotoxic treatments induces a wave of phosphorylation events targeting different substrates – e.g. histone H2A.X - 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 by a non-canonical type of ubiquitination, linked through the K27 residue of Ub. This Ub-mediated step is required for the recruitment to DDR foci of factors - 53BP1 and BRCA1 - responsible for activating downstream processes including cell cycle arrest and DNA repair.

These pioneering results open new perspectives in the field of genome stability, suggesting that specific epigenetic marks (i.e. alternative modification sites and novel Ub tags) can be used to signal detrimental cellular conditions. We are just beginning to understand how addition of these bulky peptide-based tags remodels chromatin structure to generate a specific signal alerting the cell to the presence of a harmful situation. By obtaining a clear picture of the dynamics of this novel modification we hope to identify novel markers for diagnosis and therapy of human diseases.

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DNA DOUBLE-STRAND BREAK REPAIR AND CANCER

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A major topic of research within cancer biology is the study of the causes and consequences of DNA damage at the molecular and cellular level. DNA damage not only causes cancer but is also used to treat cancer through radio- or chemotherapy and is responsible for the deleterious side effects of these treatments. DNA double-strand breaks (DSBs) induced e.g. by ionizing radiation or certain anti-cancer drugs are highly cytotoxic; their efficient and accurate repair is thus of prime importance to the cell.

The main focus of research in our laboratory is to better understand how human cells respond to DNA damage and maintain genomic integrity - an important factor in the etiology of cancer. We are particularly interested in the repair of DSBs. Because DSBs are one of the most dangerous types of lesion a cell can encounter, detailed knowledge of the factors participating in their repair and in the regulation of this process is crucial if we are to improve current cancer therapy and suggest novel strategies to fight this disease.

DSBs are repaired by two evolutionarily-conserved mechanisms: homologous recombination (HR) and non-homologous end-joining (NHEJ). The human CtIP protein plays a critical role in DSB repair pathway choice by promoting DNA-end resection and HR (Figure 1). In our laboratory we are employing several approaches to further our understanding of the regulation of CtIP function(s) and, ultimately, DSB repair by 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 gain novel insights into the molecular mechanisms of this very complex repair network.

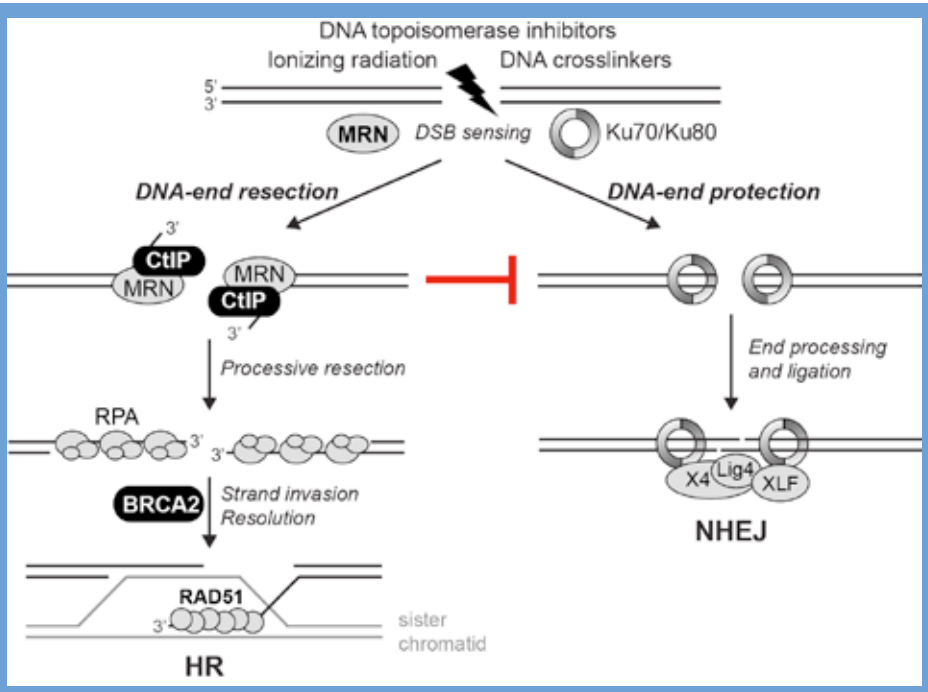

Figure 1.

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 Ku70/Ku80 heterodimer. CtIP and MRN are essential for initiating DNA-end resection, the first step in HR, and for suppressing NHEJ. The Ku70/Ku80 heterodimer drives NHEJ, which is completed by a protein complex consisting of DNA ligase IV, XRCC4 and XLF. 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.

Regulation of DNA-end resection and DSB repair by post-translational modifications

Lorenza Ferretti, Sarah Himmels

Several recent studies have indicated that CtIP function is tightly regulated by a number of post-translational modifications, including phosphorylation and ubiquitination. 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 ubiquitination and deubiquitination control CtIP function.
Using bioinformatics tools and mass spectrometry-based proteomic screens, we have recently identified several E3 ubiquitin ligases as novel CtIP interacting partners, including the APC complex/cyclosome (Figure 2) and Cullin-3. In addition, we have also identified a ubiquitin hydrolase, indicative of a dynamic balance between CtIP ubiquitination and deubiquitination. Ultimately, our goal is to identify the molecular mechanisms and cellular pathways promoting CtIP ubiquitination, and to understand their importance in the regulation of DNA-end resection and DBS repair.

Development of DSB repair inhibitors: from basic research tools to cancer treatment

Anika Trenner

Efficient DNA repair mechanisms prevent or 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 may lead to cell death. Moreover, erroneous DSB repair can result in mutations, which may, in turn, promote carcinogenesis. Conventional cancer treatment, e.g. radiotherapy or certain chemotherapeutic drugs, takes advantage of the cytotoxic properties of DSBs. However, these agents often lack selectivity for tumour cells, and the treatment often gives rise to severe side effects for the patients, thus compromising its therapeutic potential. Hence, a detailed molecular understanding of the mechanisms and factors controlling DSB repair is key to drive the development of novel compounds that display synergistic effects in combination with standard anti-cancer drugs.
The human CtIP protein promotes DNA-end resection, which is essential to direct homology-mediated repair of DSBs. Importantly, the function of CtIP in DNA-end resection is governed by multiple protein-protein interactions (PPIs). Therefore, we aim to selectively target CtIP PPI interfaces by cell-penetrating peptides (CPPs) as a novel approach to develop specific DSB repair inhibitors.
in the regulation of DNA-end resection and DBS repair.

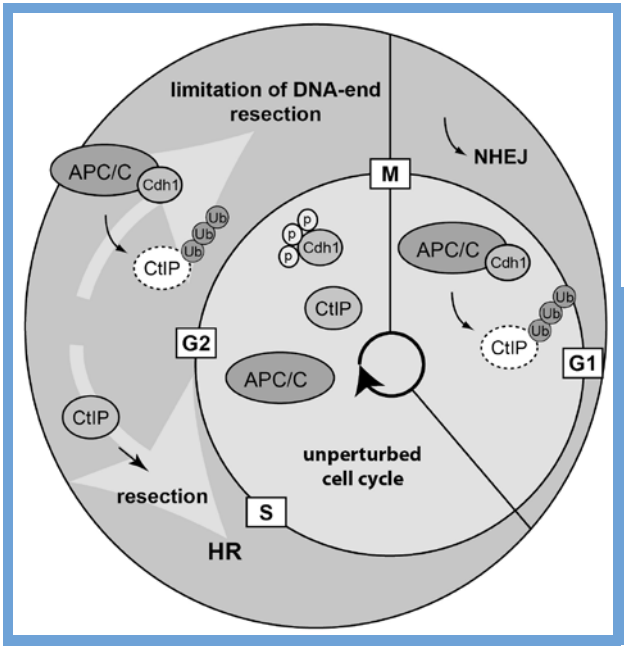

Figure 2.
Model of cell cycle-dependent regulation of CtIP by the APC^{Cdh1}. The central area represents an unperturbed cell cycle, when CtIP is degraded after mitotic exit by the APC^{Cdh1}. Cells in G_0/G_1 lack CDK activity, which precludes phosphorylation and consequent activation of CtIP. Post-mitotic CtIP degradation by the APC^{Cdh1} E3 ubiquitin ligase may help prevent unscheduled DNA-end resection in G0/G1 phase. In response to high levels of DSBs, CtIP is initially recruited to DSBs to resect DNA-ends and promote HR repair. In a late response to high levels of DNA damage, the APC^{Cdh1} promotes ubiquitin-dependent proteolysis of CtIP. Downregulation of CtIP by the APC/Cdh1 promotes its clearance from DSBs and prevents excessive DNA end-resection, a prerequisite for proper homology-directed repair.

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

Julia Godau

An accurate regulation of DNA damage response (DDR) by phosphorylation-dependent signalling is absolutely pivotal for correct repair of the DNA. Inappropriate regulation can have detrimental effects for cell fate, because incorrect repair can lead to tumorigenesis. However, surprisingly little is known about the DDR phosphorylation-network and its fine-tuning. The unique enzyme PIN1 (Peptidyl-prolyl *cis*-trans isomerase NIMA-interacting 1) acts on proteins that were previously phosphorylated on specific sites and catalyses conformational changes. A change from *cis* to trans conformation of amino acid residues in protein can have considerable consequences for its target function or stability. This post-phosphorylation action of PIN1 depicts a novel signalling mechanism. Although it might seem obvious to assume that PIN1 is involved in the DNA damage response (DDR), the implication of PIN1 in this process is not understood. Our laboratory recently identified a new role for PIN1 in the control of double-strand break repair pathway choice through promoting CtIP isomerization (Figure 3). Intriguingly, using a proteomic screen, we discovered several key DDR factors as potential substrates of PIN1. We postulate that PIN1-mediated isomerization coordinates decisive mechanisms important for the maintenance of genome stability.

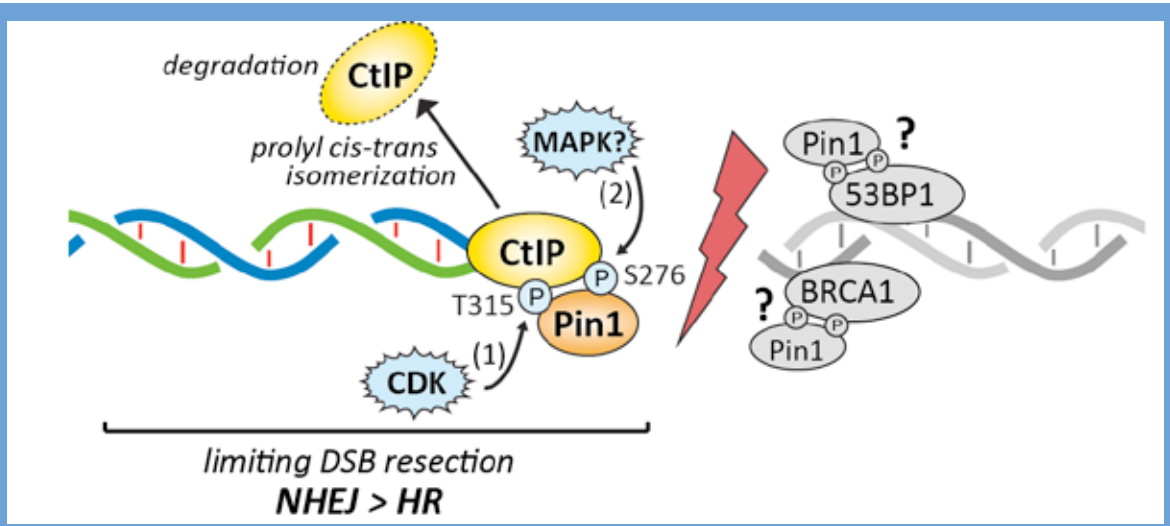

Figure 3.
Model of PIN1-mediated restriction of DNA-end resection. During S/G2 phase of the cell cycle, CtIP is phosphorylated on T315 by CDK2 and/or CDK1. This priming phosphorylation event is necessary for PIN1 binding to CtIP. Upon DSB formation, CtIP is further phosphorylated on S276 by an as-yet-unknown 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 that 53BP1 and BRCA1 could be additional PIN1 substrates. Due to their opposite roles in the regulation of DSB repair pathway choice, it will be interesting to determine whether PIN1-mediated isomerization positively (53BP1) or negatively (BRCA1) modulates their functions in NHEJ and HR, respectively.

Elucidation of synthetic lethal interactions with the multivalent adaptor protein CtIP

Hella Bolck, Sara Przetocka

In recent years, the key role of human CtIP (RBBP8) in the repair of DSBs by homologous recombination (HR) has been increasingly appreciated. CtIP initiates HR by DNA-end resection, a process that creates stretches of 3'-ssDNA overhangs at DSB ends. Furthermore, increasing evidence implicates CtIP as a critical factor for the maintenance of genome stability, owing to its roles in transcriptional regulation, DNA damage response and cell cycle checkpoint control. Recently, CtIP was shown to be recruited to stalled forks to promote their recovery and suppress new origin firing, suggesting that CtIP also participates in the response to replication stress. However, the CtIP network and the molecular mechanism of its involvement in the suppression of genome instability and the protection from tumorigenesis has been explored very little to date. In order to dissect genetic interdependencies and to investigate functional relationships of the Ctip gene, we reverted to the systematic analysis of synthetic genetic interactions of CtIP by RNA interference (RNAi)-based screening (Figure 4). We primarily focussed on aggravating, or alternatively termed, synthetic lethal/sick genetic interactions. These occur if two otherwise viable single gene disruptions lead to lethality or severely impaired cell growth (synthetic sick) when they are combined.

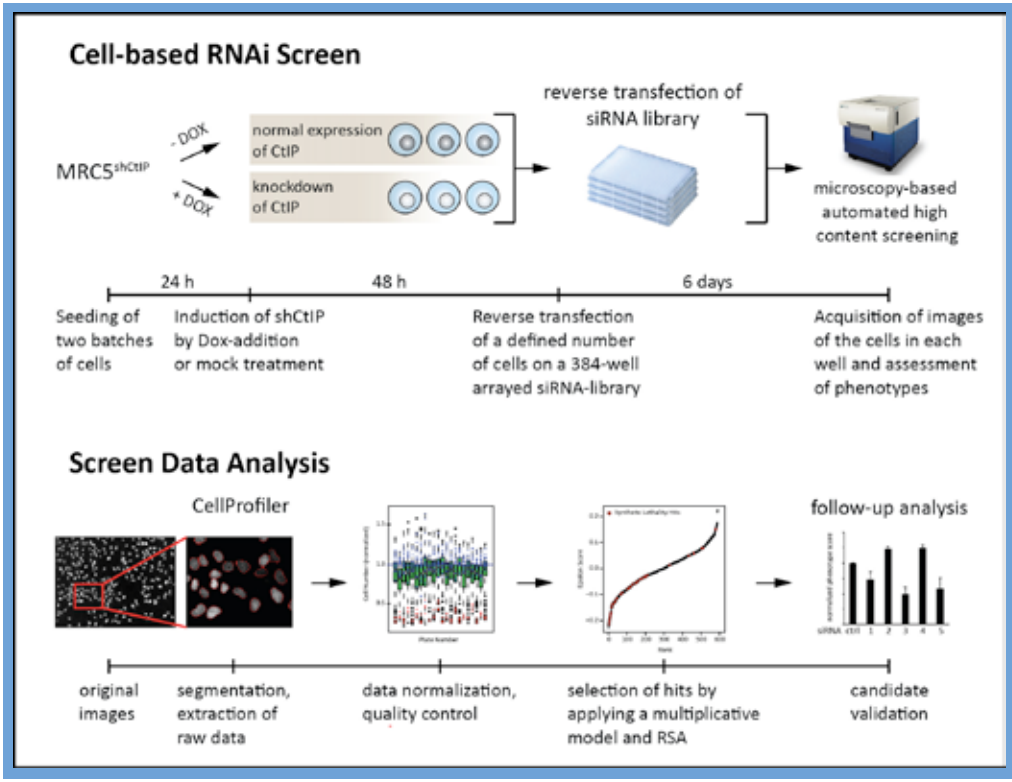

Figure 4.
Schematic overview of our cell-based RNAi screen to investigate synthetic genetic interactions of CtIP.

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This section of the Institute fulfils multiple roles. The administrators deal with personnel and financial matters, and co-ordinate the Cancer Network Zurich, the PhD Program in Cancer Biology and the University Research Priority Program in Systems Biology and Functional Genomics. The IT section updates and maintains the Institute's computer facilities and is responsible for the creation and updating of the websites of the above-named centres. The laboratory support section looks after the day-to-day running of the Institute.

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2014

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Dissertations

Ahuja Kumar, Akshay. DNA Damage Response and Replication Stress in Mouse Embryonic Stem Cells. 2014, University of Zurich, Faculty of Science.

Bologna, Serena. Post-translational modifications regulating Exonuclease 1 in response to replication forks stalling and double-strand breaks. 2014, University of Zurich, Faculty of Science.

Murina, Olga. Molecular and Cellular Aspects of DNA-end Resection by Human CtIP. 2014, University of Zurich, Faculty of Science.

Repmann, Simone. The Role of Mismatch Repair, Base Excision Repair and PARP-1 in the Processing of Oxidative DNA Damage. 2014, University of Zurich, Faculty of Science.

Uzozie, Anuli. Analysis of Proteomic Alterations during Human Colorectal Tumorigenesis. 2014, University of Zurich, Faculty of Science.

2015

Anstaett, Philipp; Pierroz, Vanessa; Ferrari, Stefano; Gasser, Gilles (2015). Two-photon uncageable enzyme inhibitors bearing targeting vectors. Photochemical & Photobiological Sciences, 14(10):1821-1825.

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Mari, Cristina; Pierroz, Vanessa; Leonidova, Anna; Ferrari, Stefano; Gasser, Gilles (2015). Towards Selective Light Activated Ru(II)-based Prodrug Candidates. European Journal of Inorganic Chemistry, 23:3879-3891.

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Pizzolato, Julia; Mukherjee, Shivam; Schaerer, Orlando D; Jiricny, Josef (2015). FANCD2-associated nuclease 1, but not exonuclease 1 or flap endonuclease 1, is able to unhook DNA interstrand cross-links in vitro. Journal of Biological Chemistry:1-20.

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Thangavel, Saravanabhavan; Berti, Matteo; Levikova, Maryna; Pinto, Cosimo; Gomathinayagam, Shivasankari; Vujanovic, Marko; Zellweger, Ralph; Moore, Hayley; Lee, Eu Han; Hendrickson, Eric A; Cejka, Petr; Stewart, Sheila; Lopes, Massimo; Vindigni, Alessandro (2015). DNA2 drives processing and restart of reversed replication forks in human cells. Journal of Cell Biology, 208(5):545-562.

Xu, Ren-Huan; Wong, Eric B; Rubio, Daniel; Roscoe, Felicia; Ma, Xueying; Nair, Savita; Remakus, Sanda; Schwendener, Reto; John, Shinu; Shlomchik, Mark; Sigal, Luis J (2015). Sequential activation of two pathogen-sensing pathways required for type I interferon expression and resistance to an acute DNA virus infection. Immunity, 43(6):1148-1159.

Zellweger, Ralph; Dalcher, Damian; Mutreja, Karun; Berti, Matteo; Schmid, Jonas A; Herrador, Raquel; Vindigni, Alessandro; Lopes, Massimo (2015). Rad51-mediated replication fork reversal is a global response to genotoxic treatments in human cells. Journal of Cell Biology, 208(5):563-579.

Dissertations

Aykut, Murat. Functional and physical interactions of 14-3-3 proteins with novel targets upon replication stress in S. cerevisiae. 2015, University of Zurich, Faculty of Science.

Engler-Anders, Daniela. Treatment with helicobacter pylorI extract protects against allergic asthma and inflammatory bowel disease. 2015, University of Zurich, Faculty of Science.

Ferretti, Lorenza P. Molecular and Cellular Aspects of CtIP Function in DNA Double-Strand Break Processing. 2015, 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.

Hartung, Mara L. Elucidating mechanisms leading to H. pylori-Induced DNA double strand breaks in host cells. 2015, University of Zurich, Faculty of Science.

Koch, Katrin N. Elucidating the mechanism of Helicobacter pylori-induced Caspase-1 activation and its role in immunity and immune tolerance. 2015, University of Zurich, Faculty of Science.

Lafranchi, Lorenzo. APC/CCdh1 Contributes to Maintenance of Genome Stability by Targeting the DNA-end Resection Factor CtIP. 2015, University of Zurich, Faculty of Science.

Levikova, Maryna. Biochemical Analysis and In Vivo Role of Dna2 Nuclease-Helicase. 2015, University of Zurich, Faculty of Science.

Pierroz, Vanessa. Photo-induced activation of metal complexes in living cells for photodynamic therapy (PDT) and photo-activated chemotherapy (PACT). 2015, University of Zurich, Faculty of Science.

Schmid, Corina A. Identification and Functional Analysis of Epigenetic Alterations in Diffuse Large B cell Lymphoma. 2015, University of Zurich, Faculty of Science.

AWARDS

Petr Cejka was awarded the Ernst Th. Jucker Prize 2015.

Hella Bolck was awarded the Charles Rodolphe Brupbacher Young Investigator Prize.

TEACHING

Cejka Petr
Genome instability and molecular cancer research
Genome stability and molecular cancer research: biochemical approaches
Mantelstudium: Cancer - From genesis to therapy
Scientific writing for PhD Students

Ferrari Stefano
Beyond central dogma: epigenetics, non- coding RNA, protein post-translational modifications and human disease
DNA metabolism and cancer
Genome instability and molecular cancer research
Molecular and cell biology of cancer
PhD Course "Cancer Biology"
Beyond the central dogma: epigenetics, protein post-translational modifications and human disease. Center for Integrative Biology (CIBIO), PhD program, University of Trento, Italy

Gari Kerstin
Genome stability and molecular cancer research: biochemical approaches

Janscak Pavel
DNA metabolism and cancer
Genome instability and molecular cancer research
Genome stability and molecular cancer research: biochemical approaches

Jiricny Josef
Arbeiten im Institut für Molekulare Krebsforschung
Cancer Research Club
Diskussion wissenschaftlicher Arbeiten
Diskussion wissenschaftlicher Projekte (Gruppenseminar)
DNA metabolism and cancer
DNS Schäden, DNS Reparatur und Krebs
Genome instability and molecular cancer research
Genome stability and molecular cancer research: biochemical approaches
Kolloquium über Literatur
Lecture Series in Molecular Life Science
Leitung wissenschaftlicher Arbeiten in Molekularer Krebsforschung für Diplomierende und Doktorierende
Molecular and cell biology of cancer
Molecular Life Sciences: Cell biology
PhD Course "Cancer Biology"
Repair, Recombination, Replication
Research Internship quantitative and systems biology
Science Ethics
Seminar in Molecular Life Sciences
Vorlesung Molekulare Zellbiologie II

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

Marra Giancarlo
Beyond central dogma: epigenetics, non- coding RNA, protein post-translational modifications and human disease
Cancer Epignetics
DNA metabolism and cancer
Genome instability and molecular cancer research
Mantelstudium: Cancer - From genesis to therapy
PhD Course "Cancer Biology"

Müller Anne
Animal Models of Cancer for PhD students in the Cancer Biology program
Cancer and the immune system
Clinical Cancer Research
DNA metabolism and cancer
Genome instability and molecular cancer research
Master Thesis in Immunology
PhD Course "Cancer Biology"
Research Internship in Immunology
Scientific writing for PhD Students
Vorlesung Molekulare Zellbiologie I
ETH Zurich - Infectious agents: From molecular biology to disease

Penengo Lorenza
Cancer Epigenetics

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

CONFERENCES

2014

Bologna Serena
Post-translational modifications of EXO1: impact on genome stability
Clare Hall Laboratories, London Research Institute, London, UK

Post-translational modifications of EXO1: impact on genome stability
Wellcome Trust/Cancer Research UK, Gurdon Institute, University of Cambridge, UK

Cejka Petr
Sae2 promotes dsDNA endonuclease of Mre11-Rad50-Xrs2 to promote 5′ DNA end resection
Abcam conference, Recombination mechanisms, Alicante, Spain

Homologous recombination: the beginning and the end
Curie Institute, Paris, France

Sae2 promotes dsDNA endonuclease of Mre11-Rad50-Xrs2 to promote 5′ DNA end resection
Faseb conference, Machines on Genes, Snowmass, CO, USA

Homologous recombination: the beginning and the end
Masaryk University, Brno, Czech Republic

Homologous recombination: the beginning and the end
National University of Galway, Ireland

Repair of damaged DNA: from mechanisms to disease therapy
School of Biomedical Sciences, University of Queensland, Brisbane, Australia

Ferrari Stefano
Post-translational modifications controlling EXO1: effect on genome stability
Department of Biology, Masaryk University, Brno, Czech Republic

Post-translational modifications controlling EXO1: effect on genome stability
EMBO-CONICET Conference: "Ubiquitin and Ubiquitin-like proteins, at the crossroads from chromatin to proteins", Buenos Aires, Argentina

Gari Kerstin
Iron-sulphur proteins and genome stability
Inaugural lecture, University of Zurich

Janscak Pavel
Exploring mechanisms of mammalian double-strand break repair
National Centre for Biomolecular Research and Department of Biology, Masaryk University, Czech Republic

Jiricny Josef
Multifaceted mismatch repair
Klenk Symposium on Molecular Medicine, Cologne, Germany

A novel insight into the contribution of polymerase proofreading and MMR to replication fidelity
DNA replication as a source of DNA damage, Casablanca, Marocco

Multifaceted mismatch repair
Swiss Congress on Genomic Instability, Weggis

Lopes Massimo
Structural and molecular insights into DNA replication stress
National Cancer Institute, NKI Amsterdam, The Netherlands

Remodelling of replication intermediates upon replication stress
Abcam "Mechanisms of Recombination, 50th Anniversary Meeting of the Holliday model", Alicante, Spain

Replication fork remodeling upon DNA replication stress in cancer onset and therapy
EMBO Young Investigator Program- Sectorial Meeting "Genome Integrity", Brno, Czech Republic

Remodelling of DNA replication intermediates in face of replication stress
FASEB Science Research Conference "Dynamic DNA Structures in Biology", Itasca, USA

Remodelling of DNA replication intermediates in face of replication stress
Fusion Conference "DNA Replication as a Source of DNA Damage", El Jadida, Morocco

Remodeling of replication intermediates in the face of DNA replication stress
Gordon Research Conference "DNA damage, Mutation and Cancer", Ventura, USA

DNA replication stress and human disease
Götz Preis 2014, University Hospital Zurich

Structural and molecular investigations into DNA replication stress in cancer onset and therapy
INsPiRE International Workshop "Oncogenic Pathways and antitumor responses", Athens, Greece

Molecular insights into DNA replication stress in cancer onset and therapy
Institute for Clinical chemistry- Klinisch-chemisches Kolloquium, University of Zurich

Replication fork remodeling upon DNA replication stress in human cells
Institute of Human Genetics, Montpellier, France

Structural and molecular investigations on DNA replication stress in cancer onset and therapy
Irish Association for Cancer Research, 2014 Annual meeting, Galway, Ireland

DNA replication stress in cancer onset and therapy
University of Messina, Italy

Müller Anne
Effective treatment of allergic asthma by tolerization with *H. pylori*-derived immunomodulators requires BATF3-dependent dendritic cell lineages and IL-10
11th International Conference on Pathogenesis and Host response in Helicobacter infections, Lo-skolen, Helsingor, Denmark

Pathogenic and immunomodulatory properties of *Helicobacter pylori* govern disease outcome in gastrointestinal and allergic disease models
98. Annual meeting of the German Society of Pathology, Berlin, Germany

Helicobacter pylori in health and disease
Institute of Rheumatology, University of Zurich

Pathogenic and immunomodulatory properties of *Helicobacter pylori* govern disease outcome in gastrointestinal and allergic disease models
Leibniz Center Infection Symposium „Pathogenesis of Infection 2014", Hamburg, Germany

Helicobacter pylori in health and disease
Max Planck Institute for Infection Biology, Berlin, Germany

Helicobacter pylori in health and disease
Symposium on Infections and Cancer, DKFZ Heidelberg, Germany

Post-transcriptional and epigenetic silencing of tumor suppressor genes in the pathogenesis of DLBCL
University of Basel, Institute of Pathology, Basel

Pathogenic and immunomodulatory properties of *Helicobacter pylori* govern disease outcome in gastrointestinal and allergic disease models
University of Erlangen, Sonderforschungsbereich Steuerungsmechanismen mikrobieller Effektoren in Wirtszellen, Germany

Helicobacter pylori in health and disease
University of Lausanne, Department of Biochemistry, Lausanne

Use of *Helicobacter pylori* or *H. pylori*-derived immunomodulators for the prevention of allergic asthma
Workshop on Microbial Intervention for Asthma – chances and challenges, Amersfoort, the Netherlands

H. pylori-specific tolerization protects against allergic airway inflammation
World Immune Regulation Meeting, Davos

Pierroz Vanessa
Photoactivatable ruthenium complexes as new anticancer agents
16th International Congress on Photobiology, Córdoba, Argentina

Sartori Alessandro
AFANCD2 recruits CtIP to promote DNA-end resection during the repair of DNA Interstrand Crosslinks
Abcam Conference, Alicante, Spain

CUL3-KLHL15 ubiquitin ligase targets CtIP for proteasomal degradation
Benzon Symposium No. 60, Copenhagen, Denmark

DNA interstrand crosslink repair: Emerging links between the Fanconi anemia pathway and homologous recombination
Department of Gynecology, University Hospital Zurich

CtIP and genome stability: New insights into the regulation of DNA-end resection
Max-Planck Institute of Biochemistry, Martinsried, Germany

DNA-end resection in human cells: New insights into the regulation of CtIP
Oxford University Biochemical Society, UK

DNA-Mutationen: eine Hauptursache für Krebs
Symposium der Krebsliga Zurich

2015

Cejka Petr
Sae2 promotes the endonuclease of MRX to initiate homologous recombination
Cancer Network Zurich Retreat (keynote lecture), Emmetten

Human MMS22L-TONSL complex promotes homologous recombination
Gordon Research Conference, Mammalian DNA repair, Ventura, CA, USA

Human MMS22L-TONSL complex promotes homologous recombination
Faseb Conference on Genetic Recombination and Genome Rearrangements, Steamboat Springs, CO, USA

Sae2 promotes the endonuclease of MRX to initiate homologous recombination
Structure-Specific Endonucleases in Genome Stability (Conference), Brno, Czech Republic

Sae2 promotes the endonuclease of MRX to initiate homologous recombination
Invited seminar, Ludwig Maximilan University of Munich, Germany

Sae2 promotes the endonuclease of MRX to initiate homologous recombination
Nordea Center for Healthy Aging, University of Copenhagen

Ferrari Stefano
The ATPases RUVBL1 and RUVBL2 as regulators of cell cycle and mitosis
Center for Integrative Biology, University of Trento, Italy

Gari Kerstin
Function and regulation of the CIA targeting complex IUBMB
Symposium FeS 2015: Iron Sulfur Cluster Biogenesis and Regulation, Bergamo, Italy

Gentili Christian
RUVBL1 inactivation leads to mitotic delay and genomic instability
Biocenter, University of Basel

RUVBL1 inactivation leads to mitotic delay and genomic instability
Molecular Biology and Genetics Meeting, Istanbul Technical University, Istanbul, Turkey

RUVBL1 inactivation leads to mitotic delay and genomic instability
Cancer Network Zurich Retreat, Emmetten

Janscak Pavel
DNA Repair and Cancer
Inaugural lecture, University of Zurich

RECQ5 DNA helicase promotes MUS81-mediated resolution of late replication intermediates during mitosis
Structure-Specific Endonucleases in Genome Stability Meeting, Brno, Czech Republic

Role of RECQ5 DNA helicase in resolution of conflicts between replication and transcription
CSHL meeting "Eukaryotic DNA Replication & Genome Maintenance", Cold Spring Harbor Laboratory, NY, USA

Jiricny Josef
Biochemical characterization of FAN1
IMB, Mainz, Germany

Biochemical characterization of FAN1
CEITEK Lecture, Brno, Czech Republic

Errors of DNA replication: their repair and consequences of their non-repair
Masaryk University Brno, Czech Republic

Personalizierte Krebsmedizin
Charles-Rodolphe Brupbacher Symposium, Zurich

Ribonucleotides misincorporated into DNA Act as strand-discrimination signals in eukaryotic mismatch repair
Keystone Symposium on Genomic Instability, Whistler, Canada

The multifaceted MMR system
Tomas Lindahl Conference, Oslo, Norway

Lopes Massimo
Fork remodeling protects mouse embryonic stem cells from endogenous replication stress during incessant replication rounds
Cold Spring Harbor Laboratory Meeting "Eukaryotic DNA replication and genome maintenance". Cold Spring Harbor, USA

Replication fork remodeling upon replication stress in cancer and stem cells
Institute of Molecular Biology, Mainz, Germany

Replication stress in cancer onset and therapy
OSTEOIMMUNE Annual Meeting & Autumn School 2015 Zurich

Marra Giancarlo
The epigenetics of colorectal tumorigenesis
University of Fribourg

Müller Anne
Helicobacter pylori in health and disease
Medizinische Hochschule Hannover, Germany

Helicobacter pylori in health and disease
ETOX meeting, Braga, Portugal

Helicobacter pylori in health and disease
Japanese Gastroenterology Conference, Sendai, Japan

Immunomodulatory activity of *H. pylori* on dendritic cells and T-cells
Leiden University Medical Center, Leiden, the Netherlands

Immunomodulatory activity of *H. pylori* on dendritic cells and T-cells
Helicobacter workshop, German Society for Hygiene and Microbiology, Herrsching, Germany

Sartori Alessandro
A novel connection between BARD1 and CtIP emerges through functional RNAi screening
EMBO Conference, Cape Sounio, Greece

CtIP ubiquitination: New insights into the regulation of DNA-end resection
Gordon Research Conference, Ventura CA, USA

COLLABORATIONS

Bangor University, Bangor, UK
DNA damage

Cancer Research UK London Research Inst., South Mimms, Hertfordshire, UK
Structural investigations of DNA replication intermediates in Xenopus egg extracts

City of Hope Helford Clinical Research Hospital, Duarte, CA, USA
RecQ helicases

CNRS Délégation Ile-de-France Sud , Gif-sur-Yvette, France
DNA damage response

Deutsches Krebsforschungszentrum, Heidelberg, Germany
DNA damage response

École Polytechnique Fédérale de Lausanne, Lausanne
Cancer stem cell biology

Erasmus University of Rotterdam, Rotterdam, The Netherlands
Mismatch repair

ETH Zürich, Zürich
DNA damage response
Colon cancer studies

IFOM-IEO, Milano, Italy
DNA replication fork

Institut Jacques Monod, Paris, France
Mismatch repair

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

IT University of Copenhagen, Copenhagen, Denmark
DNA damage response
Mismatch repair

Johannes Gutenberg-University Mainz, Mainz, Germany
Liposomes
Helicobacter pilori

Kantonsspital St. Gallen, St. Gallen
Treg in MALT lymphoma

Leopold-Franzens-Universität Innsbruck, Innsbruck, Austria
Colon cancer

Masaryk University, Brno, Czech Republic
Analysis of interaction between Mus81/Eme1 and RECQ5

Max Planck Institute for Molecular Genetics, Berlin, Germany
Helicobacter pilori

Max-Planck-Institute for Infection Biology, Berlin, Germany
Helicobacter pilori

Medizinische Hochschule, Hannover, Germany
DNA damage response

Memorial Sloan-Kettering Cancer Center, New York, USA
DNA replication

Nara Medical University, Kashihara, Nara, Japan
Isolation of DNA antibody for specific secondary structures at tri-nucleotide repeats

National Institutes of Health (NIH), Bethesda, MD, USA
Analysis of interaction between FEN1 and RECQ5

Ospedale di Cremona, Cremona, Italy
Colon Cancer

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

University of Bari, Bari, Italy
Colon cancer

University of Basel, Basel, Switzerland
Helicobacter pilori

University of Copenhagen, Copenhagen, Denmark
DNA replication
DNA resection
Cellular studies on hEXO1

University of Konstanz, Konstanz, Germany
In vitro ubiquitination of proteins

University of Kyoto, Kyoto, Japan
Cross-link repair
DNA repair

University of Lausanne, Lausanne
Colon cancer
The role of Fanconi Anemia proteins at DNA replication forks

University of Michigan-Ann Arbor, Ann Arbor, MI, USA
Colon cancer

University of Milano, Milano, Italy
Identification of specific DNA structures involved in checkpoint activation after UV damage
Cellular studies on hEXO1

University of Padova, Padova, Italy
Protein kinase inhibitors

University of Sussex, Brighton, UK
Mismatch repair

University of Trento, Trento, Italy
Protein kinase inhibitors, natural compounds

University of Trieste, Trieste, Italy
DNA repair

University of Vienna,Vienna, Austria
Mismatch repair

Wellcome Trust Sanger Institute, Cambridge, UK
Helicobacter pilori genome

SEMINARS

2014

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Myeloid cells in IL-23-driven experimental colitis

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Analysis of single cells states in disease through time and space by mass cytometry

Borde Valerie
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Controlling meiotic recombination by chromatin and chromosome structure

de Lange Titia
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How shelterin solves the telomere end-protection problem

Esashi Fumiko
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The Rad51 recombinase: how, when, where does it act?

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The SLX4 complex has SUMO E3 ligase activity that impacts on replication stress outcome

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Chromatin Replication and Epigenome Maintenance

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Recombinational DNA repair: From basic mechanism to cancer predisposition and cancer therapy

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Insights into mechanisms of breast cancer metastasis

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DNA double-strand break repair: regulation by SUMO and homology search

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RNA-guided genome defence in bacteria: structural insights and genome engineering applications

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Controlling nucleases that determine our genetic make-up

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Regulation of DSB repair and recombination during meiosis

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Replication in the vicinity of absolute blocks to replication

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Super-resolution visualization of a novel sister pairing process in DNA break repair

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Studying the dynamics of the DNA licensing complex in live cells and its involvement in tumorigenesis

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MALT1-dependent lymphoid signaling and B-cell malignancy

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Drugging the undruggable: New approaches to therapeutic inhibition of the protein tyrosine phosphatase PTP1B

2015

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Phase transitions and beyond: Image-based approaches to decipher cellular responses to genotoxic stress

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A histone variant linking metabolism and chromatin

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Mechanisms for the maintenance of genome stability

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Dissecting the molecular composition and assembly of the centriole

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Human PrimPol, bi-functional enzyme involved in DNA damage tolerance

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Molecular limits of genome integrity maintenance

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Telomere uncapping releases multiple unexpected surprises

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Tumor origins in vivo: lessons from transgenic zebrafish models

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Feedback control of cell division

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Alkylation, Inflammation and DNA Repair
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Spatial organization of DNA repair within the nucleus

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Identification of a novel factor that regulates the cellular response to damaged replication forks

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Unexpected connections between genomic instability, centrosomes and cilia

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Awakening sleeping beauty - structural & mechanistic investigation of Tc1/mariner transposons

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Mechanisms of vertebrate DNA replication and repair

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Cytoprotective signaling pathways in tissue repair and cancer

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Defective DNA strand break repair: Links to genome instability and cancer

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