SCIENTIFIC REPORT 2012/13

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FOREWORD

The Institute of Molecular Cancer Researchhas undergone further changes during 2012-2013. We have lost the group led by Reto Schwendener, who retired in the spring of 2012. Reto's group, which devoted most of its efforts to the development of liposomes as drug delivery vehicles, was one of the IMCR's main links to the clinic that resulted in numerous highly-productive collaborations. I would like to thank Reto for his contribution and support over the years.

Fortunately, laboratories do not remain empty for long at the IMCR; we were able to recruit another Swiss National Science Foundation Bursary Professor, Kerstin Gari, who joined us last September from the London Institute of Cancer Research UK. Her laboratory is already busy working on iron-sulphur cluster family of proteins, many of which are DNA repair factors. I wish her all the very best for the future.

With Kerstin's arrival, the IMCR faculty now consists of one full-, one associate- and four assistant professors, as well as three independent group leaders. These are supported by an excellent administrative and technical team, more than a dozen postdoctoral assistants and over 40 graduate students, most of whom are enrolled in the Cancer Biology PhD program.

As a double institute of the faculties of medicine and sciences, IMCR scientists have devoted a large proportion of their efforts to teaching of students of biology and medicine. The biology master course, now in its third round, is regularly oversubscribed, which confirms that our work is of substantial interest to biology students. Unfortunately, space restrictions do not allow us to extend the course to more students. IMCR scientists participated also in the teaching of other undergraduate courses, as well as in the teaching of block courses offered within the Molecular Life Sciences and Cancer Biology PhD programs of the Life Science Zurich Graduate School. IMCR scientists have also given seminars in several prominent scientific institutions in Switzerland and abroad and at national and international meetings and congresses.

IMCR scientists published a number of peer-reviewed articles that appeared in highly-reputable journals. This output was instrumental in the recruitment of top students and postdoctoral assistants, but also contributed to the successful acquisition of third party funds, primarily from the Swiss National Science Foundation, the European Community and the European Research Council. I was also extremely proud to see the outstanding work of Anne Müller and Massimo Lopes recognised by the award of the Götz Prize of the Faculty of Medicine of the University of Zurich in 2012 and 2013, respectively. Massimo also received the prestigious EMBO Young Investigator Award in 2012. With this young talent on board, the future of IMCR looks very bright indeed.

JOSEF JIRICNY

Director

IMCR CREW

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PETR CEJKA

MECHANISMS OF HOMOLOGOUS RECOMBINATION

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 the 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 in the treatment of cancer cause DNA damage and specifically DNA breaks, our work is also relevant for the understanding of the mechanisms of the mode of action of chemotherapeutics and radiation therapy.

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*left 2012

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 (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.

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

Lepakshi, Roopesh Anand

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 results in a new combination of genetic information that is different from either of the parent, contributing 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 MutLγ complex and help in producing crossovers. Despite all the genetic and cell biological data regarding Mlh1-Mlh3, mechanistic insights into its function have been lacking due to the unavailability of the recombinant protein. How crossovers can be processed into exclusively crossovers remains a key question in the recombination field. So far, we successfully expressed and purified the yeast Mlh1-Mlh3 heterodimer and showed that it is indeed an endonuclease as anticipated by genetic studies. It also binds DNA with a high affinity, and shows a marked preference for Holliday junctions. The work provides insights into the mode of Holliday junction recognition, and shows that Mlh1-Mlh3 prefers to bind the open unstacked Holliday junction conformation. Thus, the data supports the model where MutLγ is part of a complex acting on joint molecules to generate crossovers in meiosis. Currently, we are setting up experiments to elucidate the mechanism of Holliday junction resolution in vitro by MutLγ. In summary, our work is aimed to identify the mechanism leading to crossover generation in meiosis, which is a fundamental process that promotes genetic diversity in populations.

In addition, the MLH1-MLH3 complex plays a minor, yet evolutionarily conserved role in postreplicative mismatch repair. Using recombinant proteins and human cell extracts, we studying the role of MlH1-MLH3 in this pathway, and in particular the specific involvement of the nuclease activity.

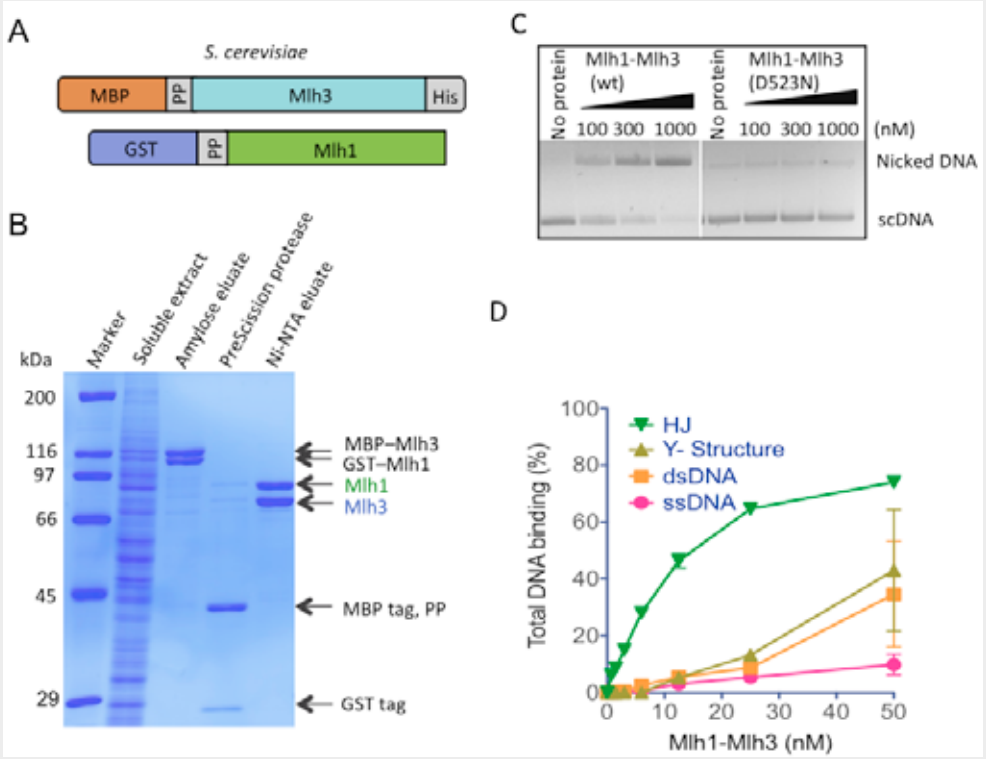

Figure 1.
The Mlh1-Mlh3 heterodimer is an endonuclease that preferentially binds to Holliday junctions.
A) A diagram of *S. cerevisiae* Mlh1 and Mlh3 constructs.
B) A representative Mlh1-Mlh3 purification showing fractions analyzed by SDS-PAGE.
C) Endonuclease assay was carried out with wild type or mutant Mlh1-Mlh3 (D523N).
D) Mlh1-Mlh3 prefers to bind Holliday junctions.
From Ranjha et al., JBC 2014 Jan 17.

The function of the 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 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 study how the interplay of both helicase and nuclease activities is regulated, and what is the biological function of the helicase activity.

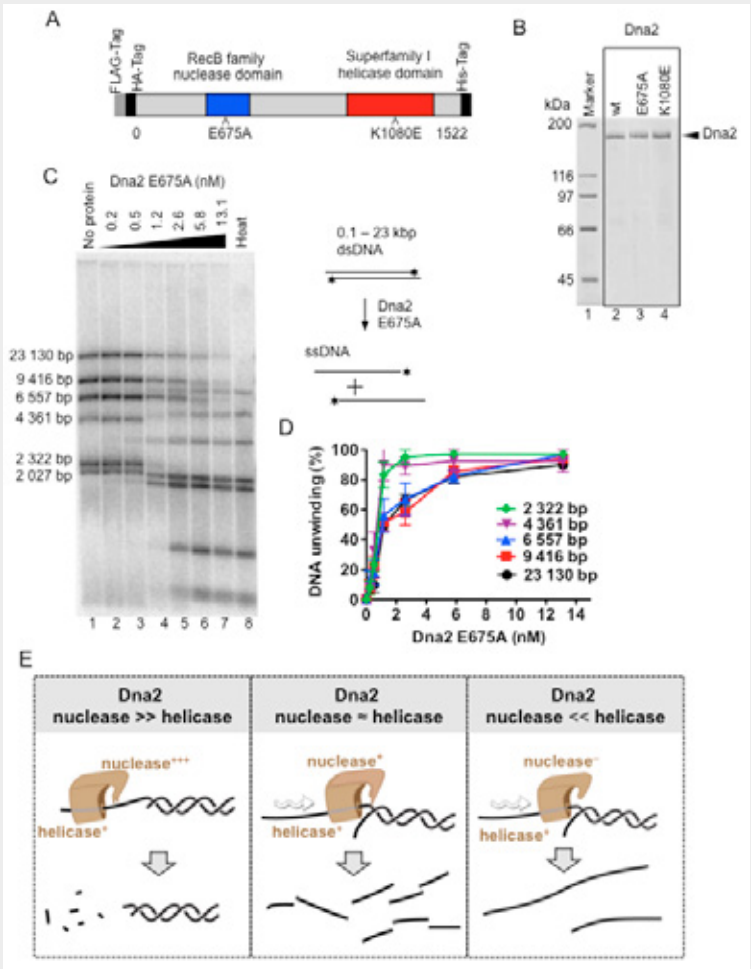

Figure 2.
Nuclease activity of *Saccharomyces cerevisiae* Dna2 inhibits its potent DNA helicase activity.
A) A schematic representation of the recombinant Dna2 protein used.
B) Purified Dna2 wild-type (wt), E675A, and K1080E variant proteins.
C) Unwinding of dsDNA fragments of 2 - 23 kbp in length by Dna2 E675A.
D) Quantitation of the helicase assays such as shown in C.
E) Model of the interplay of helicase and nuclease activities of Dna2. In the presence of vigorous nuclease activity, the helicase capacity is inhibited and the DNA degradation is limited to ssDNA (Left). Moderate inhibition of the nuclease activity might allow the enzyme to degrade dsDNA (Center). Complete inactivation of nuclease activity, such as in nuclease-dead Dna2 E675A variant, turns the enzyme into a vigorous DNA helicase (Right).
From Levikova et al., PNAS 2013 May 28 110(22).

Funding
Swiss National Science Foundation, Swiss Cancer League

STEFANO FERRARI

DNA DAMAGE AND THE CELL CYCLE

Double-strand breaks (DSBs) are the most cytotoxic lesions affecting DNA and are generated by ionizing radiation (IR), certain chemotherapeutic drugs, collapse of stalled DNA replication forks or during physiological processes such as meiotic recombination. The repair of such lesions may occur *via* error-prone or error-free pathways, depending on the phase of the cell cycle in which the lesions occur, and on initial events at sites of damage. Processing DNA lesions in an error-free manner by homologous recombination requires fine-tuning and involves several factors, nucleases among them.

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* left 2012

To elucidate the role of post-translational modifications (PTMs) in the control of DNA end-resection at sites of damage, we selected 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 that was originally identified in S. pombe and subsequently in humans. EXO1 was shown to participate in several post replication DNA repair pathways including mismatch repair and DSBs repair, as well as meiotic and mitotic recombination. More recently, yeast Exo1 was shown to localize at stalled replication forks where it counteracts fork reversal.

Focussing on EXO1 as a prototype molecule involved in the initial processing of DNA damage, we addressed the molecular mechanisms controlling its activity, localization and stability. Previous data from our laboratory indicated that EXO1 is a rare protein undergoing rapid degradation in response to stalled DNA replication (El-Shemerly et al 2005). Evidence obtained by using selective inhibitors, as well as by monitoring post-translational modifications, revealed that EXO1 protein stability is regulated through phosphorylation-dependent poly-ubiquitylation, which results in proteasome-mediated degradation (El-Shemerly et al 2005). Using chemical inhibitors, RNA interference and knock-out cell lines, we demonstrated that EXO1 is a direct target of ATR and ATR-dependent pathways. Mass spectrometric analysis allowed us to identify 12 sites of phosphorylation in EXO1, three of which are targets of DNA damage signaling pathways (El-Shemerly et al 2008). Orbitrap-based mass spectrometric analysis has recently led to the identification of more sites of phosphorylation in EXO1, the current count being 25 (Eid W., Hess D. and Ferrari S., unpublished data).

With regard to the role of EXO1 in the processing of DNA double-strand breaks (DSBs), we have addressed its functional interaction with early DNA damage sensors. We have shown that depletion of either CtIP or MRE11 impaired the recruitment of EXO1 and RPA to DSBs, indicating that the presence of EXO1 at DSBs depends on the initial end trimming carried out by MRN and CtIP. In these studies we could demonstrate that CtIP and EXO1 directly interact and that CtIP is able to restrain long-range resection by EXO1, thereby allowing the generation of recombinogenic ssDNA structures of appropriate size. Finally, we obtained evidence that CtIP and EXO1 protect cells from the deleterious consequences of end-joining-mediated repair of DSBs, thus contributing to genomic stability (Eid et al 2010).

Control of human EXO1 by post-translational modifications (PTMs)

Serena Bologna

DNA damage response proteins are tightly controlled by post-translational modifications (Bologna and Ferrari 2013). To extend our findings on the phosphorylation-dependent EXO1 degradation and clarify the molecular mechanism controlling EXO1 protein stability, we set out to identify the E2-E3 pathway responsible for EXO1 ubiquitylation. To this end, we screened an siRNA library targeting all E2-conjugating enzymes present in the human genome, and examined the fate of GFP-EXO1 by fluorescence microscopy and Western blotting. The most prominent EXO1 stabilization was obtained upon depletion of UBE2I, the human homolog of yeast Ubc9, which is the only E2 able to transfer SUMO moieties to its targets. We obtained evidence that EXO1 is SUMOylated prior to ubiquitylation and that inhibition of SUMOylation results in impaired ubiquitylation and degradation of the protein in response to DNA damage (Fig. 1). EXO1 contains four potential sites of SUMOylation: mutation of one of these sites resulted in an almost complete loss of in vitro SUMOylation. Studies conducted in cells showed altered retention of the SUMO-site mutant EXO1 at sites of damage and, accordingly, in vitro assays confirmed that non-SUMOylated EXO1 was less efficient than the SUMOylated counterpart in resecting DNA. Finally, metaphase spreads showed that the SUMO-mutant EXO1 significantly rescued the pattern of chromosomal breaks and fragmentation generated by wt-EXO1 upon treatment of cells with camptothecin.

Current focus is on the identification of the E3-SUMO ligase specifically targeting EXO1.

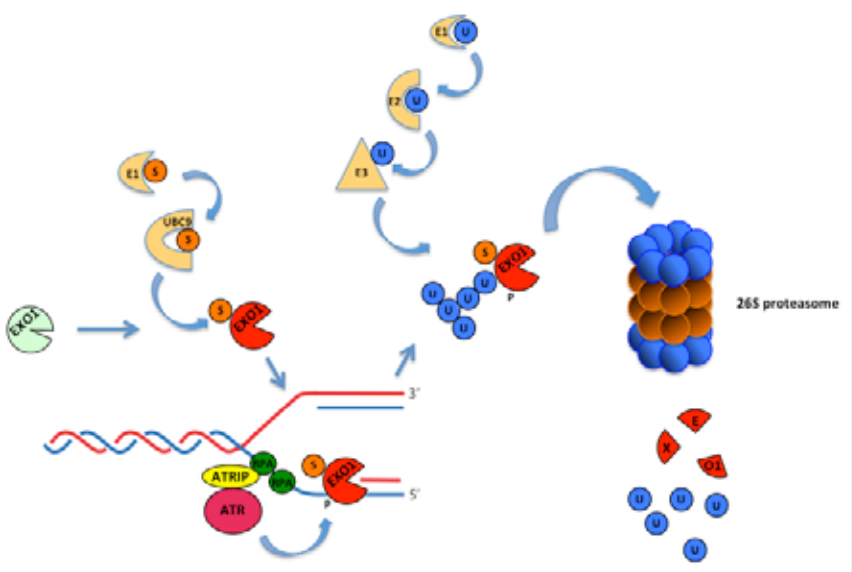

Figure 1.
PTMs controlling human EXO1 at stalled replication forks.
Upon stalled DNA replication, inactive EXO1 (light green) is SUMOylated (S) and recruited to DNA. To avoid extended, pathological resection of DNA, EXO1 is phosphorylated (P) in an ATR-dependent manner and ubiquitylated (U), thus facilitating its degradation by the proteasome.

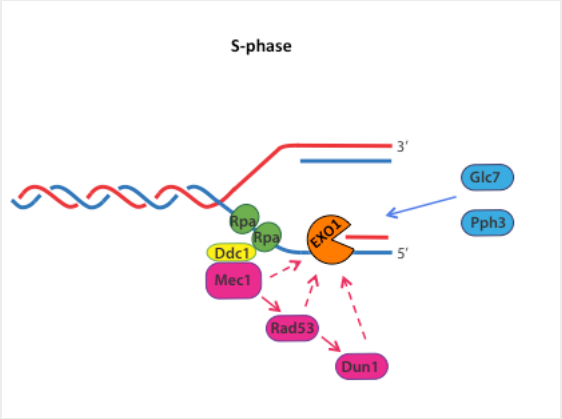

Figure 2.
Phosphorylation-dependent control of yeast Exo1 at stalled replication forks.
Upon stalled DNA replication, Exo1 is phosphorylated (P) in an Mec1/Rad53-dependent manner. During checkpoint recovery, Exo1 dephosphorylation is likely controlled by the protein phosphatases Glc7/Pph3.

Yeast Exo1: PTMs and cell cycle regulation

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. Replication fork integrity is monitored by checkpoint-mediated phosphorylation events. We have previously shown that the budding yeast Exo1 interacts and is controlled by 14-3-3 proteins in a phosphorylation-dependent manner to prevent unrestrained DNA resection at and behind stalled replication forks (Engels et al 2011). Considering that 14-3-3 proteins act as docking platforms bringing proteins into close proximity, and that they were previously described to interact with the checkpoint kinase Rad53, we set out to identify kinases and phosphatases regulating the status of Exo1 phosphorylation upon stalled DNA replication. The data so far obtained indicate that Exo1 phosphorylation in response to hydroxyurea (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).
Immunofluorescence studies have evidenced that Exo1 localizes to the nucleus during S-phase as well as in response to HU, and that Exo1 protein level fluctuates during transition through the cell cycle.

14-3-3 proteins and DNA replication

Murat Aykut

Our studies in yeast showed that 14-3-3 proteins control Exo1 activity to maintain fork integrity during stalled DNA replication. However, 14-3-3s apparently have another set of targets during checkpoint recovery and restart of replication forks (Engels et al 2011) (Fig. 3). To identify such targets, we performed mass spectrometric analysis of proteins co-precipitating with HA-tagged Bmh1 (yeast 14-3-3 protein) and compared the results with available databases listing proteins that physically or genetically interact with 14-3-3s. The major hit of our studies was a subunit of a replicative polymerase, deletion of which causes a switch from processive- to distributive-type of DNA synthesis, in a manner that apparently leads to acceleration of DNA replication forks.
Current studies are focused on the clarification of the molecular mechanism by which 14-3-3 proteins control DNA replication.

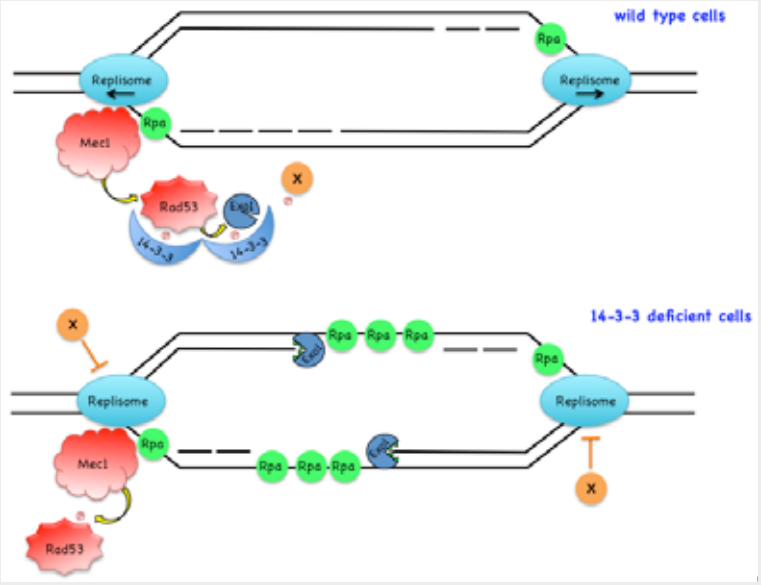

Figure 3.
Model of 14-3-3-mediated control of DNA replication. 14-3-3 proteins function as platform to facilitate the Mec1/Rad53-dependent phosphorylation of Exo1 and other unknown targets (X), contributing to the maintenance of stable replication forks (top). In 14-3-3 deficient cells, the unleashed activity of Exo1 causes the formation of ssDNA gaps behind the fork, whereas lack of control on target X impairs efficient fork restart.

Photoactivatable metal-based complexes and cancer therapy

Vanessa Pierroz

The identification of novel metal-based drugs has been recently boosted by the success of platinum-based chemotherapeutic drugs and the need for alternative compounds with less severe side-effects than those displayed by cisplatin. In collaboration with the laboratory of Prof. G. Gasser (Institute of Inorganic Chemistry, University of Zurich) we have recently examined a series of ruthenium-based complexes. Our studies led to the identification of a compound displaying increased selectivity for cancer cells over non-transformed cells that specifically targets mitochondria (Fig. 4). At the molecular level we could show that it causes an impairment of mitochondrial membrane potential resulting in apoptosis (Pierroz et al 2012). A follow-up study showed that caging such a compound with a photolabile moiety renders it innocuous to both cancer and normal cells and that its cytotoxic potential can be regained upon illumination at 350 nm (Joshi et al 2014). Recently, we identified a second ruthenium-based compound that specifically localizes to the nucleus. Light-induced activation of this compound leads to generation of a singlet oxygen, DNA cleavage and apoptosis.

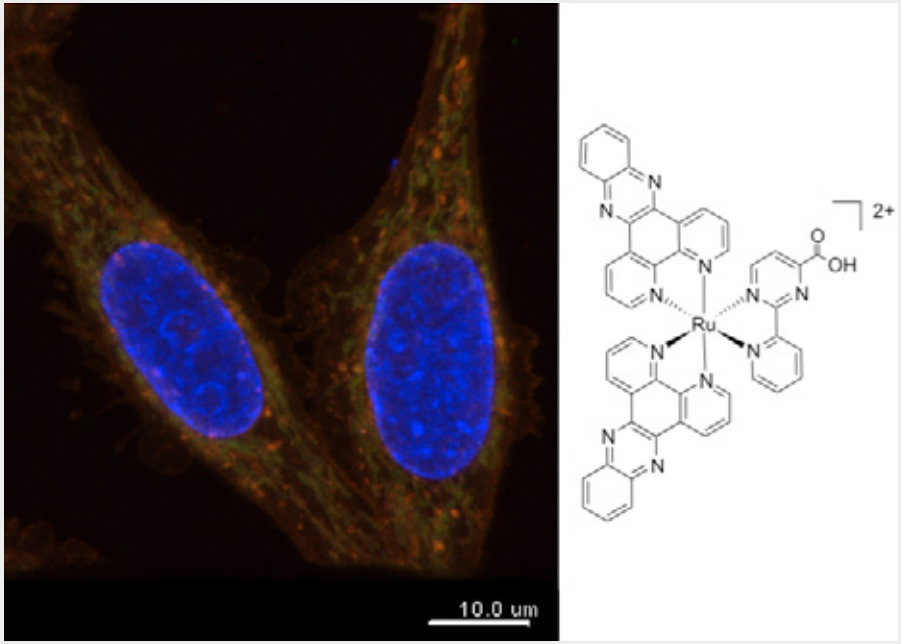

Figure 4.
Uptake of Ru-complex into mitochondria. HeLa cells were treated with 20 µM Ru-complex-1 for 2h. MitoTracker Green FM was added during the last 45 min of incubation. Cells were fixed in 4% formaldehyde and visualized using a confocal microscope SP5 (Leica). Blue: DAPI; Red: Ru-complex-1; Green: MitoTracker Green FM. The structure of Ru-complex-1 is shown on the right.

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KERSTIN GARI

IRON-SULPHUR PROTEINS AND GENOME STABILITY

In recent years, several proteins essential for DNA replication and repair have been identified to require binding to an iron-sulphur (FeS) cluster for their function. Given that upon FeS cluster oxidation, free iron can potentially generate dangerous 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 the role of FeS clusters in the maintenance of genome stability.

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The importance of genome stability

The blueprint of each cell – be it a single bacterium or part of a multi-cellular organism, such as man – is encoded in its genome. For a cell to remain functional throughout its life cycle, its genetic program has to remain accurate and, hence, its genome has to be protected from damage. Such damage can be the result of exogenous factors, such as UV light, or endogenous factors, such as the products of cellular metabolism. Moreover, the ability of a cell to duplicate its genome and divide into two identical daughter cells is a naturally complicated process that can introduce mistakes into the genetic information. To preclude these problems, cells have developed a multitude of mechanisms to protect their genomes: highly accurate factors for DNA replication, safeguard mechanisms that detect errors within the genome, and dedicated factors that can repair any possible damage within the DNA.

However, these protection mechanisms do not always work perfectly and if they fail, the consequences can be disastrous, cancer being the most renowned example. The genomes of cancer cells are intrinsically unstable, i.e. they display a high number of genetic mutations and chromosome rearrangements. As a consequence, their genetic program is altered, which can lead to uncontrolled cell growth and tumour development. Understanding in detail how genome stability is maintained is therefore an important step towards understanding cancer development.

Iron-sulphur proteins

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 (Figure 1).

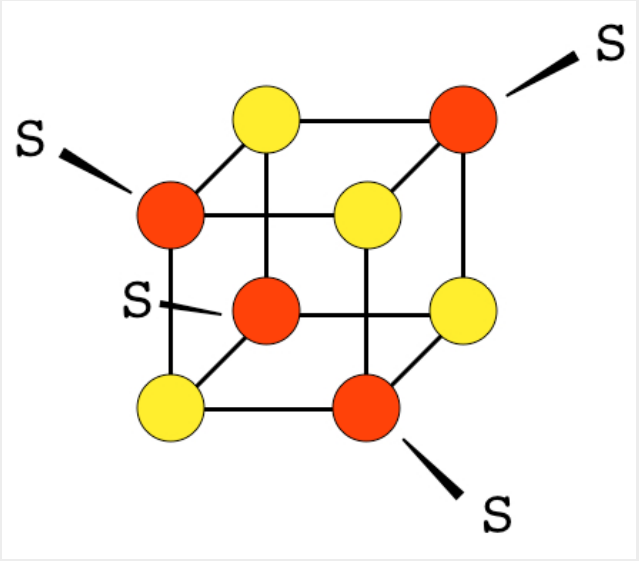

Figure 1.
Schematic of a [4Fe-4S] cluster. Iron atoms are depicted in red, sulphur atoms in yellow. S denote cysteinyl ligands that coordinate the cluster.

Iron-sulphur proteins in DNA replication and repair

For a long time it appeared that nuclear FeS proteins with affinity for DNA are relatively rare. Given that upon FeS cluster oxidation, free iron atoms can generate reactive oxygen species and potentially damage DNA, this notion seemed rather intuitive.

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.

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 2).

In the absence of MMS19, when FeS protein maturation is impaired, the stability of FeS proteins and their assembly into functional complexes is affected. However, the actual function of FeS clusters in DNA replication and repair proteins has remained largely elusive to date.

Owing to their redox sensitivity, FeS clusters are particularly interesting and versatile cofactors that would be uniquely suited as regulatory cofactors that could e.g. sense oxidative stress conditions during DNA replication. One attractive possibility is that FeS clusters could serve as intrinsic stress sensors within DNA replication and repair proteins and directly modulate their biochemical activities to allow adaption to suboptimal conditions of DNA replication.

We are going to use a combination of techniques, such as biochemistry, molecular biology and structural studies, in order to address the function of FeS clusters in DNA replication and repair.

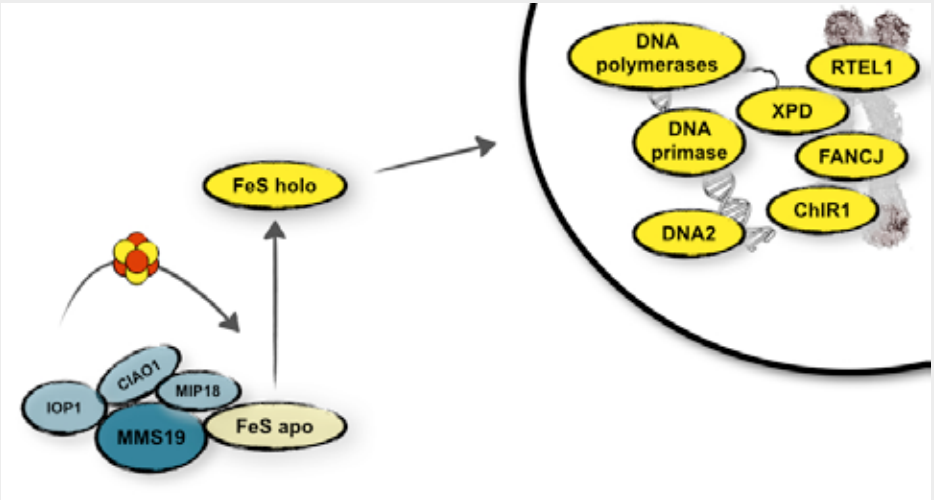

Figure 2.
MMS19-MIP18 links cytoplasmic iron-sulphur cluster assembly to DNA metabolism.

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Swiss National Science Foundation, Human Frontiers Science Program, URPP Systems Biology/Functional Genomics

PAVEL JANSCAK

RECQ DNA HELICASES AND GENOME STABILITY

DNA damage is a frequent event in the life of a cell. Failure to repair DNA damage can lead to cell death, while inaccurate DNA repair can give rise to genomic instability, which promotes the onset of cancer in mammals. The research in our laboratory focuses on understanding the molecular mechanisms of DNA repair processes in human cells. Specifically, we combine biochemical and cell biological approaches to study the cellular functions of RecQ DNA helicases that play a critical role in the maintenance of genomic stability and have a proven tumor suppressor function. Our recent studies provide new mechanistic insights into the function of these proteins in repair of DNA double-strand breaks (DSBs) and in the processing of stalled replication forks.

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* left 2012/13

Role of RECQ5 helicase in the regulation of homologous recombination

Shreya Paliwal, Andreas Sturzeneger

Homologous recombination (HR) is an evolutionarily conserved process that facilitates chromosome segregation during meiosis and eliminates DNA DSBs arising accidentally in S/G2 phase of the cell cycle. Most mitotic HR events proceed via a synthesis-dependent strand annealing (SDSA) mechanism to avoid crossing-over, which may give rise to chromosomal rearrangements and loss of heterozygosity. However, the molecular mechanisms controlling HR sub-pathway choice are poorly understood. We have a long-standing interest in deciphering the role of the human RECQ5 DNA helicase in the regulation of HR. RECQ5 possesses the unique ability to disrupt RAD51-ssDNA filaments that perform homology search and strand exchange to form the so-called D-loop structures, branch points in the choice of HR sub-pathways. Our recent studies using cell-based reporter assays have revealed that RECQ5 specifically promotes the formation of non-crossover products during HR-mediated repair of endonuclease-induced double-strand breaks (DSBs), suggesting a role for RECQ5 in SDSA (Figure 1). We have also found that RECQ5 counteracts the inhibitory effect of RAD51 on RAD52-mediated ssDNA annealing *in vivo* and *in vitro*. Moreover, our studies have indicated that depletion of RECQ5 leads to increased binding of RAD51 to chromatin flanking a DSB site. Finally, we have found that lack of RECQ5 causes an elevation of sister chromatid exchanges upon inactivation of the Holliday junction dissolution pathway or upon induction of a high load of DNA damage in human cells. Collectively, these findings suggest that RECQ5 acts during the post-synaptic phase of SDSA to prevent formation of aberrant RAD51 filaments on the extended invading strand, thus limiting its channelling into the potentially-hazardous crossover pathway of HR. We are currently interested in understanding the mechanisms involved in the regulation of RECQ5 function in the process of HR-mediated DSB repair.

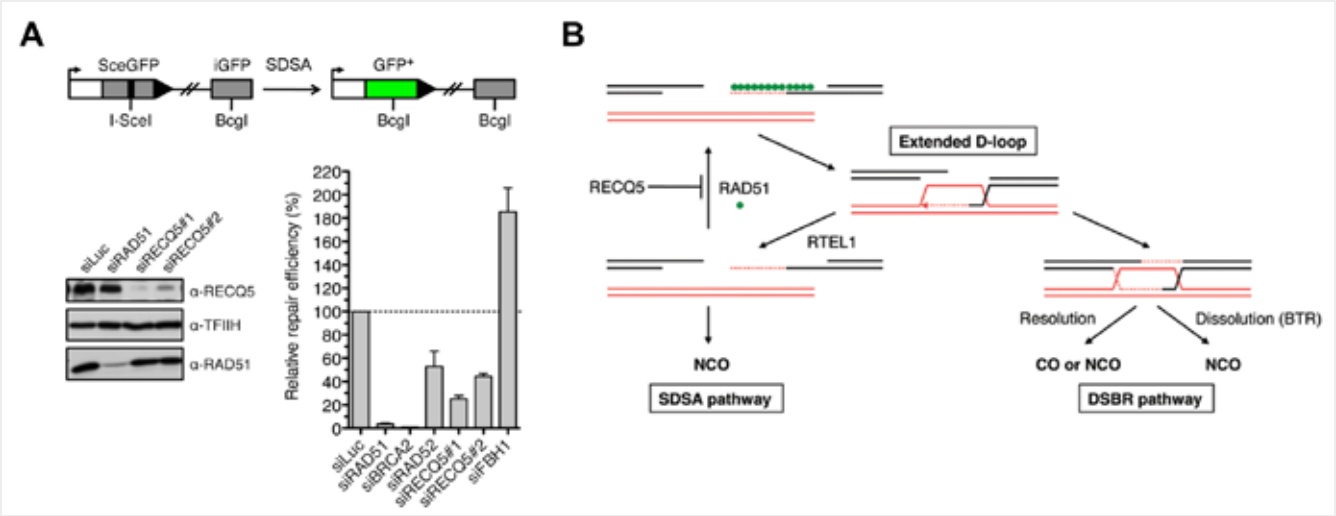

Figure 1.
RECQ5 helicase promotes repair of DNA double-strand breaks by synthesis-dependent strand annealing. (A) RECQ5 depletion reduces the efficiency of HR-mediated repair of endonuclease induced-DSBs as determined by DR-GFP reporter assay. The site-specific DSB in the chromosomally integrated reporter cassette is generated by I-*SceI* endonuclease. The functional GFP allele formed indicates non-crossover (NCO) events. (B) Model for the roles of RECQ5 and BLM in suppression of crossovers (COs) during DSB repair by HR. RECQ5 promotes SDSA by disrupting aberrant RAD51 filaments formed after unwinding of the extended D-loop. BLM acts as a part of the BTR (BLM-TOPOIIIα-RMI1/2) complex to mediate dissolution of double-Holliday junctions.

Role of RECQ5 in processing of stalled replication forks

Stefano Di Marco, Naga Raja Chappidi, Shruti Menon

Replication machinery frequently stalls at bulky DNA lesions or at various natural impediments such as unusual secondary DNA structures or oncoming transcription complexes. Failure to stabilize and properly process stalled replication forks can result in genomic instability. The human genome contains regions, termed common fragile sites (CFSs), that are difficult to replicate and have a propensity to form gaps or breaks on metaphase chromosomes, especially upon partial inhibition of DNA synthesis. These loci are frequently damaged in precancerous lesions and are hotspots for chromosomal rearrangements associated with tumour progression. Interestingly, recent studies have shown that the breakage of CFSs is an active process that takes place in prometaphase and is mediated by the MUS81-EME1 heterodimer, a structure-specific endonuclease that can cleave a replication fork structure at the base of the leading arm. The MUS81-mediated sister chromatid disjunction at CFSs prevents the formation of the so-called ultrafine anaphase bridges, which can lead to an uneven distribution of DNA between the daughter cells. Our recent studies have revealed that MUS81 forms a complex with the RECQ5 helicase in human cells (Figure 2A). Moreover, we have found that RECQ5 directly binds to the MUS81-EME1 complex and stimulates its fork cleavage activity *in vitro*. Our *in vivo* experiments have shown that RECQ5 is required for MUS81-dependent chromosome breakage induced by Chk1 inhibition, which abrogates the cellular response to replication stress (Figure 2B). Interestingly, using cell cycle inhibitors, we have found that DSBs caused by Chk1 inhibition are generated in early mitosis where MUS81-EME1 is activated for cleavage of CFSs. We now aim to explore the possible role of RECQ5 in MUS81-mediated resolution of late-replication intermediates during mitosis. We are also studying the role of RECQ5 in MUS81-mediated cleavage and reactivation of replication forks stalled by topoisomerase-DNA complexes.

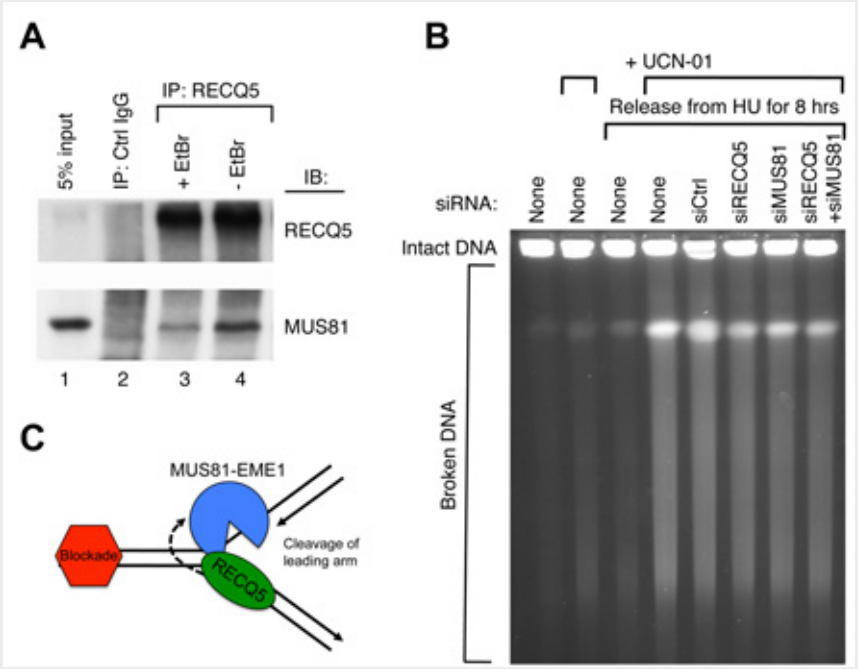

Figure 2.
Physical and functional interaction between RECQ5 and MUS81.
(A) Co-immunoprecipitation of MUS81 with RECQ5 from human cell extracts.
(B) RECQ5 and MUS81 are required for the formation of DSB in response to replication stress induced by Chk1 inhibition with UCN-01. The integrity of genomic DNA was analyzed by pulse-field gel electrophoresis.
(C) Working model. RECQ5 is proposed to stimulate cleavage of stalled replication forks by MUS81-EME1.

Role of RecQ helicases in DNA-end resection

Andreas Sturzeneger

The 5′-3′ resection of DNA ends is a prerequisite for repair of DNA DSBs by HR, microhomology-mediated end joining (MMEJ) and single-strand annealing (SSA). Recent studies in yeast have shown that following an initial 5′-strand processing by the Mre11 complex and Sae2, extension of resection tracts is mediated either by Exonuclease 1 (Exo1) or by combined activities of the RecQ-family DNA helicase Sgs1 and the helicase/endonuclease Dna2. Although the human DNA2 was shown to cooperate with the BLM helicase to catalyze resection of DNA ends *in vitro,* the identity of RecQ DNA helicase that acts in conjunction with DNA2 to mediate DNA-end resection in mammalian cells remains unclear. Our recent studies have shown that both BLM and WRN act epistatically with DNA2 to promote SSA-mediated repair of endonuclease-induced DSBs, providing evidence that not only BLM but also WRN can act in concert with DNA2 to resect broken DNA ends in cells. In line with this assumption, our biochemical experiments have shown that WRN and DNA2 interact physically and coordinate their activities to mediate extensive resection of 5′-ends of DNA in a reaction dependent on RPA and ATP (Figure 3). Interestingly, by measuring chromatin binding of RPA around an I-SceI-induced DSB, we found that depletion of either DNA2 or one of the two RecQ helicases dramatically increased the EXO1-dependent long-range resection activity in cells. These findings suggest that DNA2 in conjunction with WRN or BLM restrains DNA-end resection by EXO1 and is preferentially used for DNA DSB repair. We are now attempting to obtain further mechanistic insights into the long-range DNA-end resection processes in human cells.

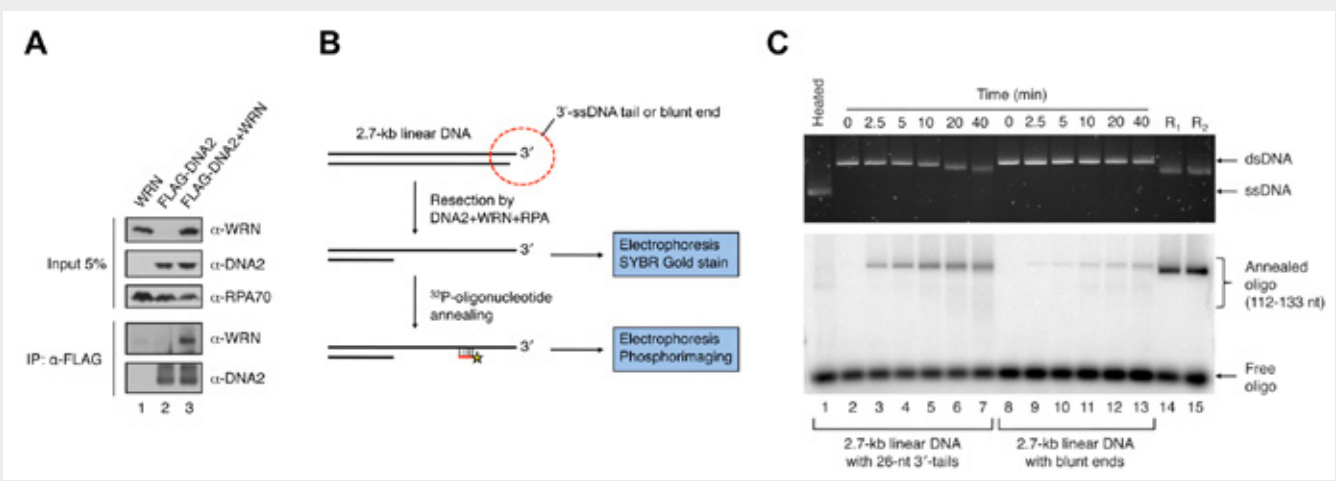

Figure 3.
DNA2 resects DNA ends in conjunction with WRN helicase.
(A) Co-immunoprecipitation of RECQ5 with Flag-tagged DNA2 from human cell extracts. (B) Scheme of *in vitro* DNA-end resection assay. (C) Time course of resection of 3′-tailed and blunt-ended DNA substrates by DNA2 and WRN in the presence of RPA and ATP.

Funding
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JOSEF JIRICNY

THE MULTIFACETED MISMATCH REPAIR

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, we have been trying to understand how MMR functions and how its malfunction leads to malignant transformation. However, evidence emerging from several different directions implicated MMR proteins also in other pathways of DNA metabolism and we are now changing direction and concentrating our efforts on some of these processes, mostly in human systems. We have also begun to explore the potential of other systems, specifically the DT40 chicken bursal B cells.

We are also revisiting the field of DNA demethylation, with a specific focus on the molecular mechanism of the process and its effects on different pathways of DNA metabolism.

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Biochemistry of mismatch repair

Medini Ghodgaonkar

To improve replication fidelity, MMR must detect non-Watson-Crick base pairs and direct their repair to the nascent DNA strand. Eukaryotic MMR *in vitro* requires pre-existing strand discontinuities for initiation; consequently, it has been postulated that MMR *in vivo* initiates at Okazaki fragment termini in the lagging strand, and at nicks in the leading strand generated by the mismatch-activated MLH1/PMS2 endonuclease. We were able to show that a single ribonucleotide in the vicinity of a mismatch can act as an initiation site for MMR in human cell extracts and that MMR activation in this system is dependent on RNase H2 (Figure 1). As loss of RNase H2 in *S. cerevisiae* resulted in a mild MMR defect that was reflected in increased mutagenesis, MMR in vivo might also initiate at RNase H2 generated nicks. We therefore proposed that ribonucleotides misincoporated during DNA replication serve as physiological markers of the nascent DNA strand (Ghodgaonkar et al., 2013).

The minimal MMR system could be reconstituted from purified proteins some time ago (Constantin et al., 2005; Zhang et al., 2005), but our genetic screens (Cejka and Jiricny, 2008) and proteomic analysis of the MMR interactome (Cannavo et al., 2007) identified several polypeptides that strongly associate with MLH1 and PMS2, yet are apparently not required for minimal MMR.

In order to learn whether the identified interactors play accessory role(s) in MMR, we set up the reconstituted system in our laboratory, in addition to the *in vitro* assay that makes use of nuclear extracts of human cells.

We have also set out to study the involvement of nucleases in human MMR. The rationale for this work is the finding that the mutator phenotype of MSH2- and MLH1-deficient cells is considerably stronger than that of cells lacking EXO1, the only exonuclease implicated in MMR to date. This suggests that additional nucleases compensate (at least partially) for the lack of EXO1. One of the candidate nucleases we have been studying is 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 (Figure 2). Phenotypic analysis of these cells revealed that all three cell lines had substantially elevated mutation frequencies, which implies either that their MMR capacity was saturated, or that MMR does not address errors that escape the proofreading exonuclease.

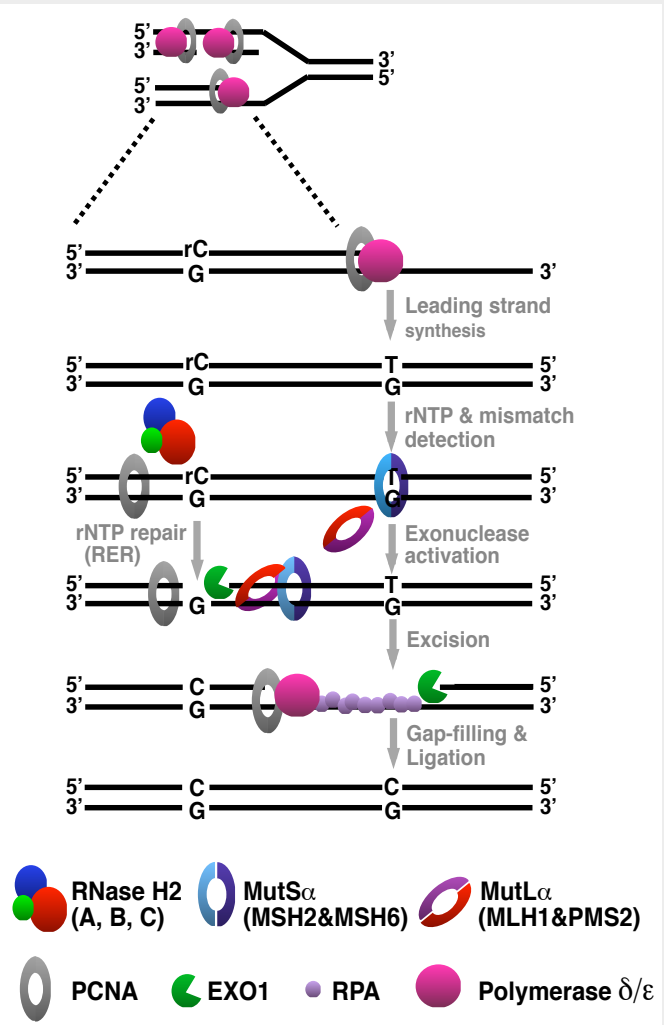

Figure 1.
Schematic representation of ribonucleotide-directed mismatch repair. Ribonucleotides incorporated into the nascent strand during replication are removed by RNasH2. Should a mismatch be generated in the vicinity, the MMR system can hijack the strand break arising during the ribonucleotide removal as an initiation site for EXO1-catalysed degradation of the error-containing nascent strand. The single-stranded gasp is stabilised by RPA, until it is filled-in by the replicative polymerase.

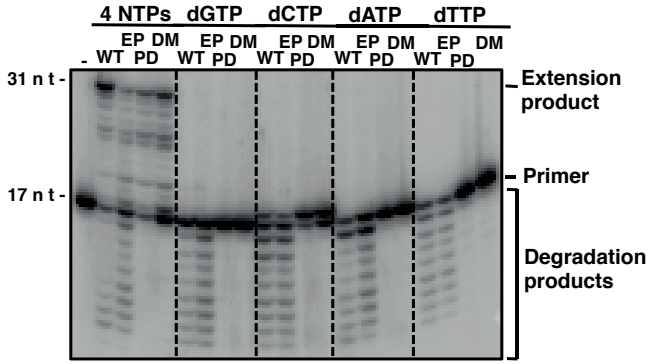

Figure 2.
Biochemical characterization of polymerase-δ variants expressed in human cells. The enzymes were isolated by affinity chromatography and tested for their ability to extend a radiolabelled 17-mer primer annealed to a 31-mer template. All enzyme variants, wild type (WT), error-prone (EP), proofreading-deficient (PD) or the double mutant (DM) could extend the primer in the presence of all four dNTPs, albeit with varying efficiencies. The exonuclease defect in the PD and DM variants is clearly evident from the lack of degradation products of the 17-mer. All variants were able to incorporate dCMP (but no other nucleotide) opposite the G at position 18 of the template. The figure represents an autoradiograph of a 10% polyacrylamide gel.

MMR and interstrand cross-link repair

Svenja Kaden, Julia Pizzolato

In 2007, we identified KIAA1018 as a strong interactor of the MMR protein MLH1. Because the protein is recruited to chromatin by mono-ubiquitylated *Fanconi* protein FANCD2, it has been renamed FANCD2-associated nuclease 1, FAN1. We could show that FAN1 is an exo/endonuclease, which preferentially cleaves 5′ flaps and D-loops *in vitro*. We were able to show that human (Kratz et al., 2010) and chicken DT40 (Yoshikiyo et al., 2010) cells lacking FAN1 were hypersensitive to agents that induce interstrand cross-links (ICLs), and that FAN1 deficiency also lowered recombination efficiency and double-strand break repair. Interestingly, although hypersensitivity to ICL-inducing agents is one of the key hallmarks of *Fanconi anemia* (FA), the *FAN1* gene does not appear to be mutated in FA patients. We are now trying to understand the biological relevance and/or importance of the binding of FAN1 to MLH1 and PMS2, as well as identify its molecular role in the processing of ICLs.

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

Maite Olivera Harris

The MMR system has been shown to be involved in the processing of DNA damage other than base/base mismatches and IDLs. It is 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 S_N1 type than their MMR-proficient counterparts. In order to understand the molecular basis of this resistance, we asked whether DNA substrates carrying defined base modifications are addressed by the MMR system *in vitro*. We devised a method of preparing such substrates, using a combination of primer extension reactions on single-stranded substrates and a "nickase" – an enzyme capable of incising specifically only a single DNA strand of its recognition sequence. We succeeded in incorporating ^{Me}G into our substrates, and were able to show that it is addressed by the MMR system. However, our *in vitro* MMR assay does not faithfully mirror the process in which a mispair is addressed immediately after it was generated by the polymerase, i.e. in the context of DNA replication. In order to gain insights into the mechanism of postreplicative MMR, we are attempting to make use of ^{Me}G present in the template that is undergoing replication in nucleoplasmic extracts of *Xenopus laevis* eggs. In this system, we can follow replication, repair, DNA damage signalling and possibly also recombination in the same assay.

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The role of MMR proteins in antibody diversification

Stephanie Bregenhorn, Javier Peña-Diaz, Lia Kallenberger

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. This interference gives rise to long tracts of single-stranded DNA, which are not efficiently filled-in by the replicative polymerases due to low enzyme concentrations and depleted nucleotide pools outside of S phase. This appears to trigger mono-ubiquitylation of PCNA and recruitment to DNA of translesion polymerases such as polymerase-η. We postulate that the deployment of these error-prone polymerases in the repair of MMR-generated gaps leads to mutations during SHM/CSR (Figure 3).

We are currently attempting to obtain mechanistic insights into the CSR process, using an in vitro system capable of mimicking the AID-triggered *in vivo* recombination events.

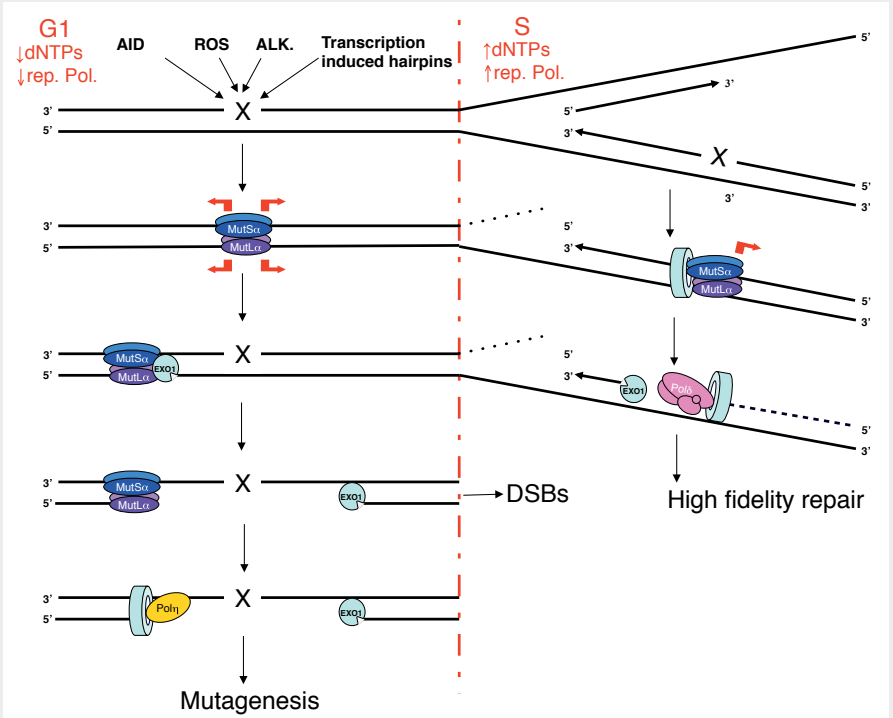

Figure 3.
Putative scheme of MMR function during G1- and S-phases of the cell cycle. Lesions bound by MMR proteins outside of S-phase activate non-canonical MMR, during which the endonuclease activity of MutLα introduces nicks into either DNA strand. These might be used for loading of EXO1, which would result in the generation of long single-stranded gaps. Due to low nucleotide pool concentrations and low levels of replicative polymerases, the gaps might persist for some time, which could trigger PCNA ubiquitylation and recruitment of error-prone polymerase(s) such as pol-η. In contrast, lesions generated during S-phase would be repaired with high fidelity, due to the existence of free termini that direct MMR to the nascent strand, the ready availability of dNTPs and higher concentrations of replicative polymerases.

MASSIMO LOPES

DNA REPLICATION STRESS AND HUMAN DISEASE

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, which contribute to cancer, aging and a growing number of neurodegenerative human syndromes. 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 (DNA fiber spreading, psoralen-crosslinking coupled to electron microscopy).

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

Arnab Ray Chaudhuri, Marko Vujanovic

DNA replication interference is one of the most common strategies employed in the clinic to kill actively-proliferating cancer cells. Topoisomerasel (Top1) can be trapped by specific inhibitors, such as Camptothecin or its clinically relevant derivatives Topotecan and Irinotecan, leading to interference with DNA metabolism and resulting in potent cytotoxicity in proliferating and cancer cells. Although replication-induced DSB have been consistently proposed to mediate this cytotoxicity, several recent reports challenge this view and propose a more complex coordination of replication fork progression in face of the topological stress induced by Top1-inhibition. Our single-molecule-, biochemical- and genomic studies in *S. cerevisiae*, mammalian cells and *Xenopus* egg extracts show that Top1 poisons rapidly induce replication fork slowing and reversal (Figure 2), which can be uncoupled from DSB formation at sublethal doses. Poly (ADP-ribose) polymerase activity, but not single strand break repair in general, is required for effective fork reversal and limits DSB formation. These data identify fork reversal as a cellular strategy to prevent chromosome breakage upon exogenous replication stress and provide novel means to identify cellular factors that limit or mediate the cytotoxicity of anticancer drugs inducing replication stress. We now plan to test the contribution of specific cellular factors likely to play a role in the formation, remodelling and/or resolution of reversed forks. We are particularly interested in testing *in vivo* the role of nuclease and helicase activities previously suggested to form or restart regressed forks. Among these we aim to identify PARP target proteins, as this could potentially explain the role of PARP in replication fork remodelling in the face of stress. We are also assessing how the fine-tuning of Poly-ADP-ribosylation (via PARP and its antagonist protein PARG) contributes to fork structure and resistance to genotoxic stress. Furthermore, it will be particularly important to assess whether fork reversal is a specific response to Top1 poisoning, or whether it entails a more general DNA transaction upon treatment with a wide range of cancer chemotherapeutics.

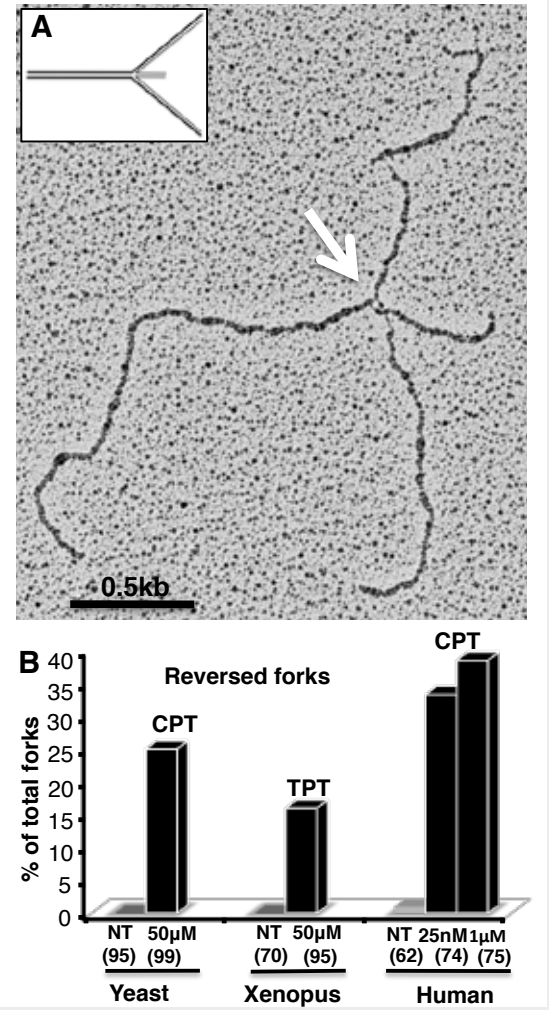

Figure 1.
Top1 poisoning results in replication fork reversal. (A) Representative electron micrograph and schematic drawing of a reversed fork observed on genomic DNA from CPT-treated U2OS cells. The white arrow points to the four-way junction at the replication fork. (B) Frequency of fork reversal in yeast (*S. cerevisiae*) cells (50μM CPT, 30min), *Xenopus* egg extracts (50μM TPT, 50 min) and U2OS cells (25nM or 1μM CPT, 1h). In brackets, the number of analyzed molecules. NT, not treated.

Structural insights into oncogene-induced DNA replication stress

Kai Neelsen, Sofija Mijic

TDNA 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. 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 about the DNA structures generated in vivo under such conditions.

Replication stress phenotype can be reproduced in cell culture by overexpression of various oncogenes influencing DNA replication, e.g. *Cyclin E* or *Cdc25A*. We have exploited these systems to identify oncogene-associated defects in DNA replication. Overexpression of both oncogenes has a substantial effect on bulk DNA synthesis and leads to a marked slow-down of individual replication forks, measured by FACS analysis and DNA fiber labelling, respectively. Furthermore, electron microscopic analysis reveals the accumulation of aberrant replication intermediates upon oncogene induction. However, only the overexpression of Cdc25A causes massive DNA breakage and full DDR activation shortly after oncogene induction (Figure 1). We found that Cdc25A-dependent DNA double strand breaks (DSB) are suppressed by preventing mitotic entry. We therefore propose that oncogene-induced replication stress promotes the accumulation of unusual replication intermediates and that oncogene-dependent DSB arise due to premature activation of mitotic factors. Using a similar set of approaches we have also recently characterized the molecular consequences of "re-replication", a deregulation of a replication initiation program that is frequently associated with tumorigenesis. We are now in the process of extending our studies to a broad spectrum of oncogenes, in an attempt to identify common molecular mechanisms underlying tumorigenesis. Furthermore, we will test the involvement of known cancer susceptibility factors in these molecular processes, as we suspect that altered cellular responses to replication stress could underlie the high incidence of cancer associated with certain genetic defects.

Structural analysis of DNA replication across unstable repetitive sequences

Cindy Follonier, Katharina Zwicky, Judith Oehler

A growing number of human neurological hereditary diseases - among which Huntington disease, Freidreich's Ataxia and Fragile-X Syndrome are the most prominent - have been associated with trinucleotide repeat (TNR) expansion at various genomic loci. A large body of evidence suggests that these events are associated with DNA replication interference. Extensive studies in bacteria and yeast showed that TNR can cause pausing of DNA replication forks. Non-B DNA structures - such as hairpins, slipped DNA structures, triplexes, or "sticky" DNA - have been shown to form *in vitro* at TNR-containing sequences and excellent correlation has been found between the length of the repeated tracts required to adopt such structures and the length found in carriers and patients of the corresponding disease. Nonetheless, compelling evidence is still missing on which structures form in human cells and contribute to TNR instability during DNA replication.

We established a plasmid-based system to recover abundant human replication intermediates and combined gel electrophoresis and electron microscopy to study *in vivo* fork structure and progression across GAA repeats. We found that replication forks pause transiently and reverse at expanded GAA tracts in both orientations. Furthermore, we identified replication-associated intramolecular junctions involving GAA and other homopurine-homopyrimidine tracts, which we link to pausing and breakage of the sister plasmid fork not traversing the repeats. Finally, we show postreplicative, sister-chromatid hemicatenanes on control plasmids to be converted into persistent homology-driven junctions at expanded GAA repeats (Figure 3). Overall, these data provide novel insights into how premutation GAA tracts interfere with replication and suggest new working hypotheses for trinucleotide repeat expansion. We now plan to combine the powerful investigation system described above with genetic tools (siRNAs), to test the role of candidate mammalian factors in the formation/resolution of the recently-identified GAA-specific structures and, more in general, in the stability of repetitive tracts during replication.

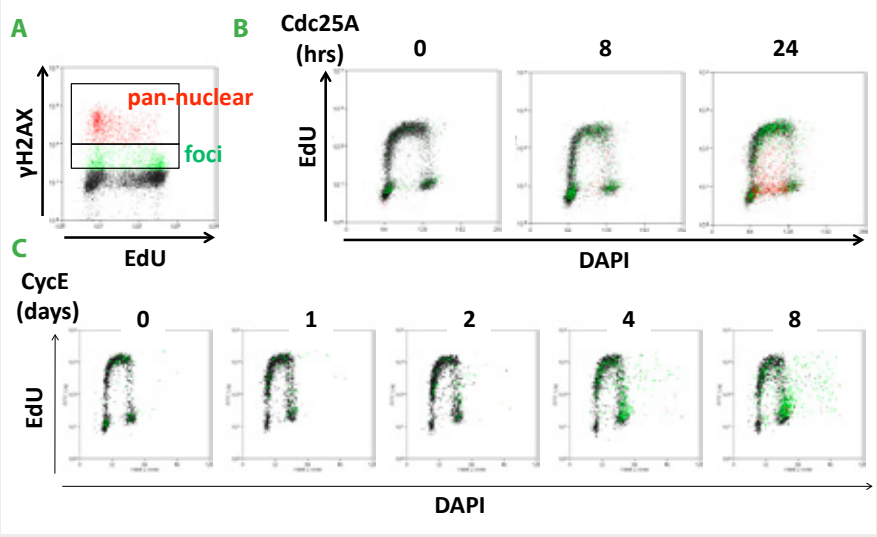

Figure 2.
Flow cytometric analysis of DNA synthesis, cell cycle progression and DNA damage response activation after oncogene expression. (A) FACS-based distinction of γH2AX patterns after Cdc25A induction. Red and green signals indicate cells with pan-nuclear γH2AX and γH2AX foci respectively, indicative 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) FACS analysis after CycE induction shows early S-phase accumulation, followed by accumulation of cells in G2/M and checkpoint activation. At late timepoints, re-replicating cells with ≥4n DNA are detectable.

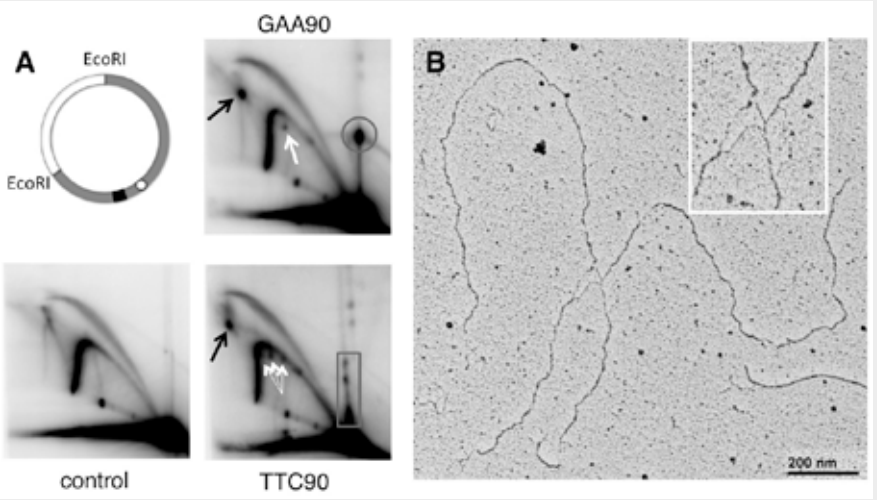

Figure 3.
Expanded GAA/TTC repeats induce unusual replication intermediates in human cells. (A) Neutral-neutral 2D-gel analysis of plasmids containing the indicated numbers of GAA or TTC repeats as template for lagging strand synthesis. Plasmids were transfected in 293T cells, recovered after 40h, digested by EcoRI (A), processed by 2D-gel and probed with the fragment depicted in gray. In the map: circle, SV40 origin; black square, GAA/TTC repeats. Intermediates specific to GAA/TTC repeats are indicated. Black arrow: "2N-spot"; white arrow(s): "Y-spot(s)"; gray circle/rectangle: "1N-spot(s)". (B) Representative electron micrograph of a molecule migrating in the gel area of the 2N spot from GAA90 plasmid EcoRI-fragment. Magnification 46kx.

A potent technological platform to support structural DNA investigations worldwide

Thanks to the rare combination of specialized approaches, our laboratory has also been very actively involved in worldwide collaborative efforts requiring structural insights into DNA metabolism. A number of projects have been published in high-impact journals, while several other collaborative efforts are either ongoing or approaching the publication stage.

DNA replication stress in stem cells?

Akshay Ahuja

Embryonic stem cells (ESCs) have the unique ability to self-renew and are capable of differentiating into multiple cell types. Therefore, ESCs need to constantly cope with the need to populate any given niche. In contrast, exhaustion of many adult stem cells - haematopoietic stem cells (HSCs) in particular - has been linked to ageing, but the underlying molecular mechanisms are 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 recently started to investigate the intriguing connection between replication stress and ageing in different populations of stem cells (ESCs and HSCs) by a variety of techniques available in the laboratory. We found that ESCs contain numerous sub-nuclear foci of the endogenous DNA damage marker γH2AX, which markedly diminish upon induction of differentiation (Figure 4), when the differentiating cells are still actively dividing. Hence, stemness seems inherently associated with genotoxic stress. Interestingly, ESCs lack 53BP1 foci, but exhibit strong staining for RPA and Rad51, suggesting that the observed DDR activation results from perturbations of the replication process, rather than DNA breakage. Similarly, HSCs undergoing replication under standard conditions - or upon interferon α-induced proliferation - show DDR activation and markedly reduced rate of nucleotide incorporation. We now aim to take advantage of our most specialized methods - DNA fiber spreading and electron microscopy - to characterize in more detail this putative replication stress in stem cells. These studies could significantly advance our knowledge of how ESCs proliferate rapidly while maintaining their genome stability; also, they could shed light on the cellular mechanisms leading to stem cell exhaustion in ageing individuals.

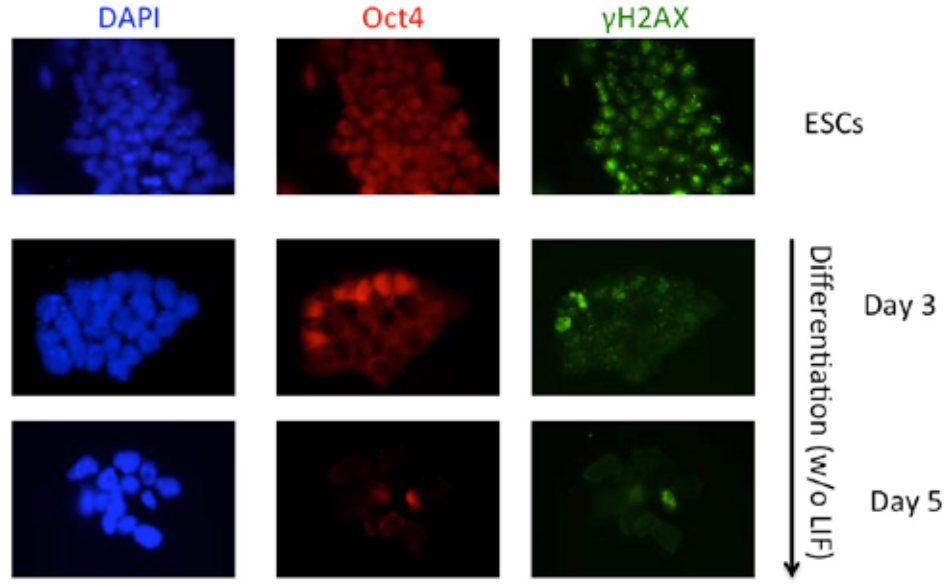

Figure 4.
Kinetics of ESC differentiation reveals that γH2AX is lost upon the onset of differentiation: ESCs accumulate high levels of γH2AX (marker of DNA damage response), which is gradually lost upon induction of differentiation, in parallel to loss of the stem cell marker Oct4, but well before they stop undergoing replication.

Funding
Swiss National Science Foundation, Krebsliga Zürich, Krebsliga Schweiz, Promedica/UBS, European Research Council, URPP Systems Biology/Functional Genomics

GIANCARLO MARRA

COLON CANCER

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.

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* left 2012/13

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.

Preinvasive colorectal lesion transcriptomes correlate with endoscopic morphology (polypoid vs. nonpolypoid)

E. Cattaneo , E. Laczko, F. Buffoli, F. Zorzi, M.A. Bianco, M. Menigatti, Z. Bartosova, R. Haider, B. Helmchen, J. Sabates-Bellver, A. Tiwari, J. Jiricny, G. Marra

Improved colonoscopy is revealing precancerous lesions that were frequently missed in the past, and ~ 30% of those detected today have nonpolypoid morphologies ranging from slightly raised to depressed. To characterize these lesions molecularly, we assessed transcription of 23,768 genes in 42 precancerous lesions (25 slightly elevated nonpolypoid and 17 pedunculated polypoid), each with corresponding samples of normal mucosa. Nonpolypoid versus

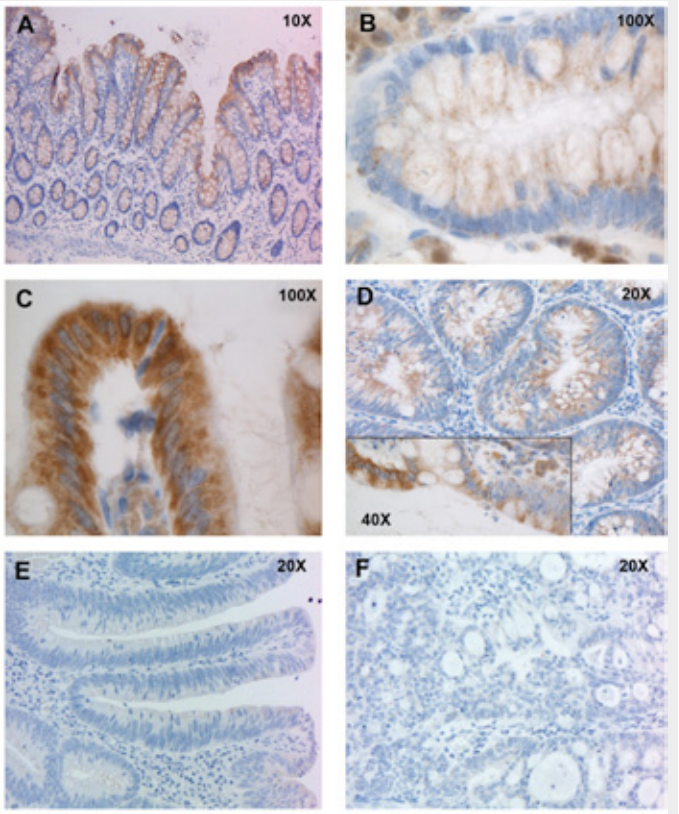

Figure from EMBO Mol Med 2011; 3:334-347

polypoid morphology explained most gene expression variance among samples; histology, size, and degree of dysplasia were also linked to specific patterns. Expression changes in polypoid lesions frequently affected cell-cycling pathways, whereas cell-survival dysregulation predominated in nonpolypoid lesions. The latter also displayed fewer and less dramatic expression changes than polypoid lesions. Paradigmatic of this trend was progressive loss through the normal > nonpolypoid > polypoid > cancer sequence of TMIGD1 mRNA and protein. This finding, along with TMIGD1 protein expression patterns in tissues and cell lines, suggests that TMIGD1 might be associated with intestinal-cell differentiation. We conclude that molecular dysregulation in slightly elevated, nonpolypoid, precancerous colorectal lesions may be somewhat less severe than that observed in classic adenomatous polyps.

Risk of colorectal cancer in men on long-term androgen deprivation therapy for prostate cancer

S. Gillessen, A. Templeton, G. Marra, Y.F. Kuo, E. Valtorta, V.B.Shahinian

Androgen deprivation with gonadotropin-releasing hormone (GnRH) agonists or orchiectomy is a common but controversial treatment for prostate cancer. Uncertainties remain about its use, particularly with increasing recognition of serious side effects. In animal studies, androgens protect against colonic carcinogenesis, suggesting that androgen deprivation may increase the risk of colorectal cancer.

We identified 107 859 men in the linked Surveillance, Epidemiology, and End Results (SEER)-Medicare database who were diagnosed with prostate cancer in 1993 through 2002, with follow-up available through 2004. The primary outcome was development of colorectal cancer, determined from SEER files on second primary cancers. Cox proportional hazards regression was used to assess the influence of androgen deprivation on the outcome, adjusted for patient and prostate cancer characteristics. All statistical tests were two-sided.

Men who had orchiectomies had the highest unadjusted incidence rate of colorectal cancer (6.3 per 1000 person-years; 95% confidence interval [CI] = 5.3 to 7.5), followed by men who had GnRH agonist therapy (4.4 per 1000 person-years; 95% CI = 4.0 to 4.9), and men who had no androgen deprivation (3.7 per 1000 person-years; 95% CI = 3.5 to 3.9). After adjustment for patient and prostate cancer characteristics, there was a statistically significant dose-response effect (P(trend) = .010) with an increasing risk of colorectal cancer associated with increasing duration of androgen deprivation. Compared with the absence of these treatments, there was an increased risk of colorectal cancer associated with use of GnRH agonist therapy for 25 months or longer (hazard ratio [HR] = 1.31, 95% CI = 1.12 to 1.53) or with orchiectomy (HR = 1.37, 95% CI = 1.14 to 1.66).

We conclude that long-term androgen deprivation therapy for prostate cancer is associated with an increased risk of colorectal cancer.

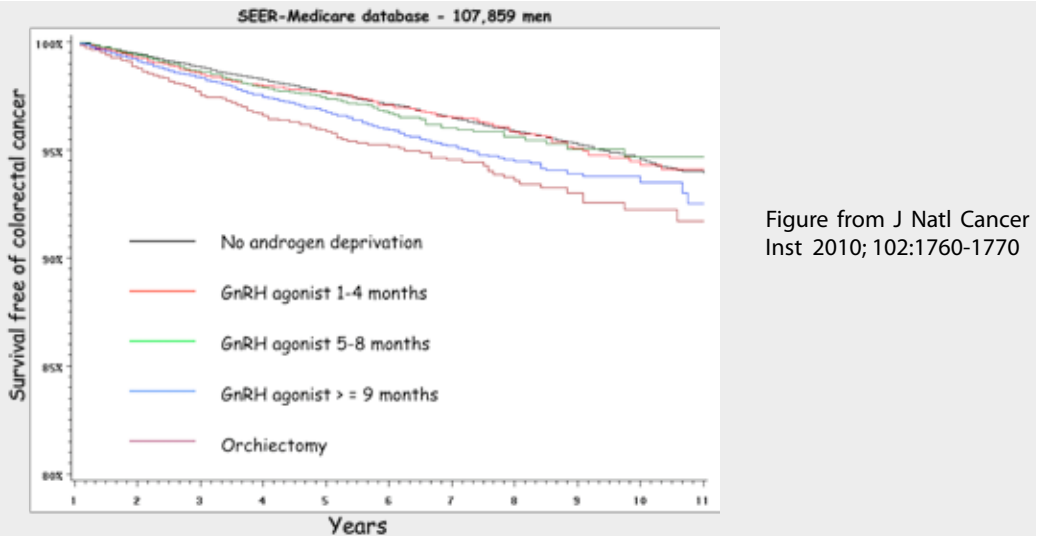

Figure from J Natl Cancer Inst 2010; 102:1760-1770

Familial colorectal cancer: eleven years of data from a registry program in Switzerland

M. Kovac, E. Laczko, R. Haider, J. Jiricny, H. Mueller, K. Heinimann, G. Marra

Deleterious germ-line variants involving the DNA mismatch repair (MMR) genes have been identified as the cause of the hereditary nonpolyposis colorectal cancer syndrome known as the Lynch syndrome, but in numerous familial clusters of colon cancer, the cause remains obscure. We analyzed data for 235 German-speaking Swiss families with nonpolyposis forms of colorectal cancer (one of the largest and most ethnically homogeneous cohorts of its kind) to identify the phenotypic features of forms that cannot be explained by MMR deficiency. Based on the results of microsatellite instability analysis and immunostaining of proband tumor samples, the kindreds were classified as MMR-proficient (n = 134, 57%) or MMR-deficient (n = 101, 43%). In 81 of the latter kindreds, deleterious germ-line *MMR*-gene variants have already been found (62 different variants, including 13 that have not been previously reported), confirming the diagnosis of Lynch syndrome. Compared with MMR-deficient kindreds, the 134 who were MMR proficient were less likely to meet the Amsterdam Criteria II regarding autosomal dominant transmission. They also had primary cancers with later onset and colon-segment distribution patterns resembling those of sporadic colorectal cancers, and they had lower frequencies of metachronous colorectal cancers and extracolonic cancers in general. Although the predisposition to colorectal cancer in these kindreds is probably etiologically heterogeneous, we were unable to identify distinct phenotypic subgroups solely on the basis of the clinical data collected in this study. Further insight, however, is expected to emerge from the molecular characterization of their tumors.

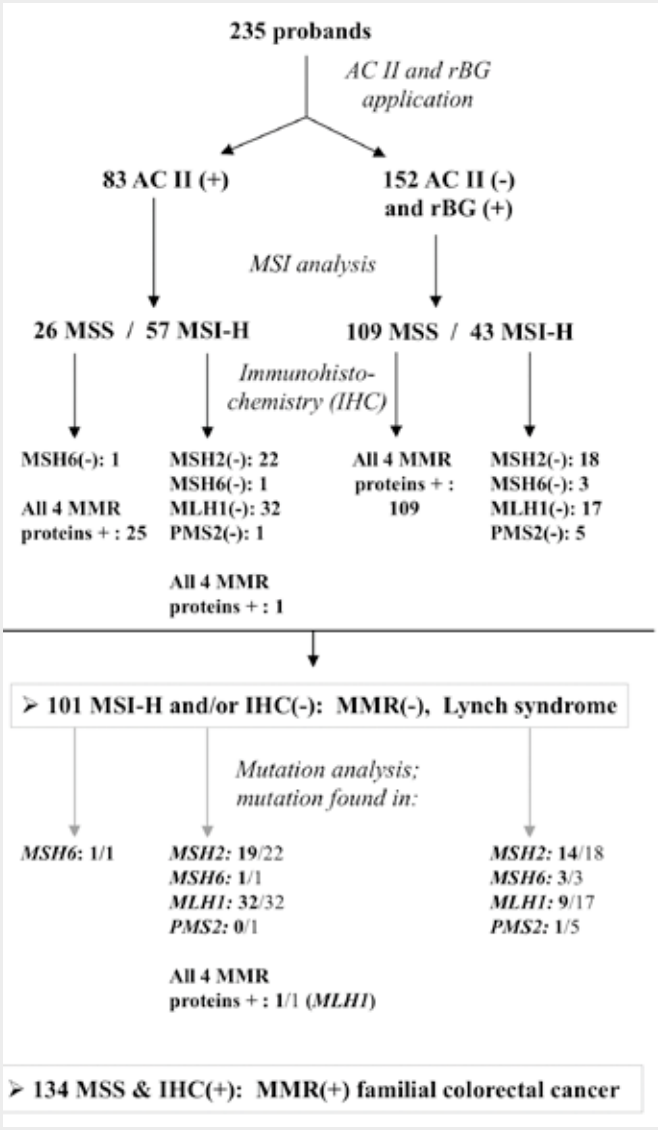

Figure from Familial Cancer 2011; DOI 10.1007/s10689-011-9458-6

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

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

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.

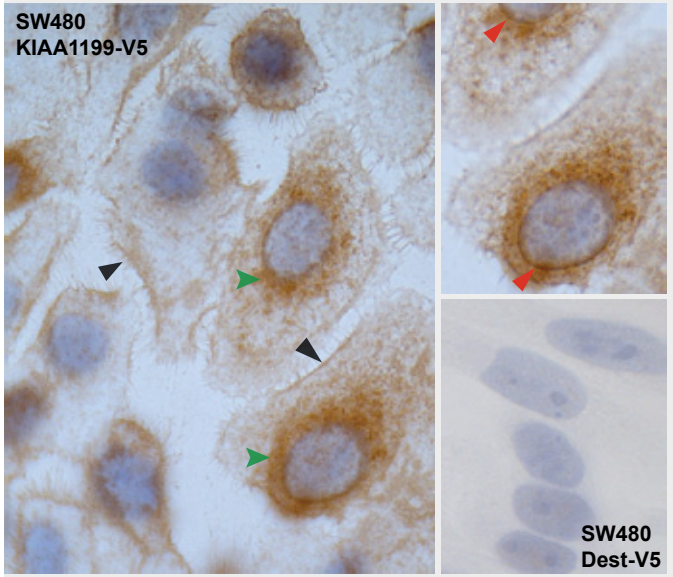

Figure.
Immunocytochemical staining of SW480 colon cancer cells with or without expression of KIAA1199. In SW 480 KIAA1199-V5 cells, KIAA1199 is localized in the cell membrane (gray arrowheads) and perinuclear space (green arrowheads). Upper right inset: Fine focusing clearly reveals staining of the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

Funding
Swiss National Science Foundation, Swiss Cancer League, URPP

ANNE MÜLLER

HELICOBACTER PYLORI AND GASTRIC CANCER

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.

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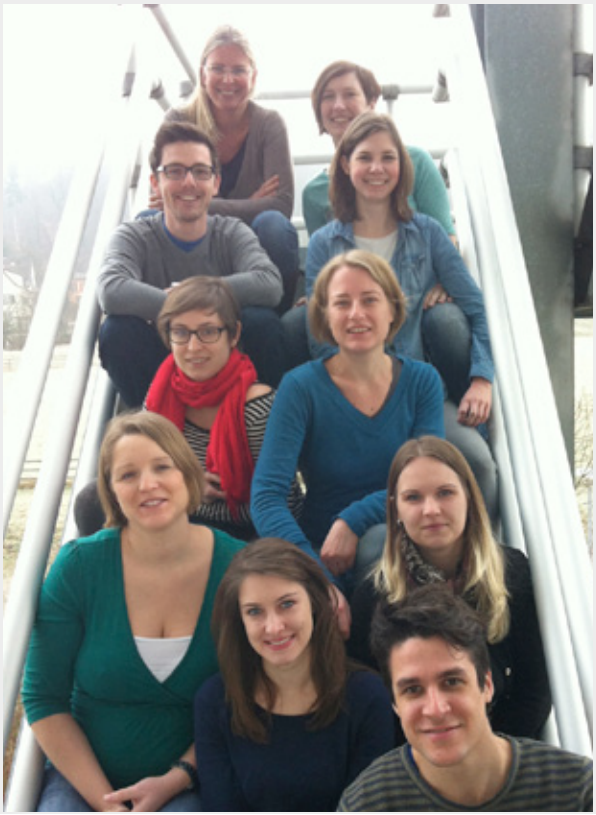

Mechanisms of immune tolerance induction by *H. pylori* and of systemic immunomodulation

Mathias Oertli, Daniela Engler, Sabine Urban, Andreas Kyburz

One of the most exciting discoveries of our recent work was that the outcome of the *Helicobacter*/host interaction differs depending on the age of the host at the time of first exposure. If we infect mice during the neonatal period (i.e. at a time when *H. pylori* is typically transmitted from mothers to their babies in regions where *H. pylori* is endemic), they fail to control the infection (i.e. they are colonized at 50-100 fold higher levels), but are completely protected against the gastric immunopathology that is a hallmark of adult-infected mice (Arnold, 2011c). This relative resistance to *H. pylori*-associated disease despite heavy colonization is maintained for at least one year, i.e. it is not restricted to the newborn period. A closer examination of the mechanism of protection revealed that neonatally infected mice preferentially generate *H. pylori*-specific Treg- over T-effector responses, and develop immune tolerance to the infection (Arnold, 2011c). The systemic depletion of Tregs breaks this tolerance, and leads to clearance of the bacteria and severe gastric pathology (Arnold, 2011c). We believe that similar processes may be operative in humans infected as children, and may explain the findings of high Treg/Teff ratios in the gastric mucosa of infected children (Harris, 2008), and the lack of *H. pylori*-associated stomach problems in certain areas of the world where *H. pylori* is endemic (and presumably transmitted early in life).

Based on a series of papers describing an inverse epidemiological association between *H. pylori* infection and asthma and other allergic disease manifestations, especially in children and young adults (Blaser, 2008; Chen, 2007; Chen, 2008; Reibman, 2008), we hypothesized that immune tolerance to *H. pylori* might cross-protect against allergen-specific, pathogenic T-cell responses. Using an experimental model of allergic airway disease induced by ovalbumin-specific sensitization and challenge, we found that *H. pylori* infection protects mice against the clinical and histopathological symptoms of asthma, i.e. airway hyper-responsiveness, tissue inflammation and goblet cell metaplasia, and prevents the infiltration of eosinophils, Th2- and Th17-cells into the bronchoalveolar fluid and lungs (Arnold, 2011a). As predicted, the protection is largely restricted to neonatally infected mice; the results could further be reproduced also with an allergen (house dust mite antigen) relevant in humans (Arnold, 2011a). Most strikingly, asthma protection could be transferred from neonatally infected to naive mice via small numbers of highly purified regulatory T-cells, which we isolate from the gut-draining lymph nodes of the infected donors. Conversely, the depletion of Tregs abrogates asthma protection (Arnold, 2011a). Taken together, the results indicate that neonatally acquired immune tolerance to *H. pylori* not only prevents the gastric immunopathology that underlies and precedes *H. pylori*-associated gastric disease, but may also be beneficial in preventing asthma (summarized in Figure 1).

To elucidate the mechanisms involved in the induction and maintenance of immune tolerance to *H. pylori*, we are focussing primarily on dendritic cells (DCs), as these cells are known to exhibit tolerogenic (as well as immunogenic) properties in the gut (Maldonado, 2010). Indeed, we found that *H. pylori* has evolved to effectively re-program DCs towards a tolerance-promoting state; contact of DCs with *H. pylori* generates so-called "semi-mature" DCs that express high levels of MHCII, but no or low amount of co-stimulatory molecules such as CD80 and CD86 (Oertli, 2012). *H. pylori*-experienced DCs also do not express T-cell-activating cytokines such as IL-12, but preferentially produce IL-10 (Oertli, 2012). DCs that have been exposed to *H. pylori in vitro* or *in vivo* furtherfail to induce T-cell effector functions, and instead efficiently induce expression of the Treg lineage-defining transcription factor FoxP3 in naive T-cells. The experimental depletion of DCs breaks *H. pylori*-specific, neonatally acquired tolerance and results in improved control of the infection, but also in aggravated immunopathology. DCs infiltrating the gastric mucosa of human *H. pylori* carriers exhibit a semi-mature DC-SIGN^{+}HLA-DR^{high}CD80^{lo}CD86^{lo} phenotype, indicating that a human cell counterpart exists for our observations in the experimental model (Oertli, 2012). Interestingly, the tolerogenic activity of *H. pylori*-experienced DCs requires interleukin-18 *in vitro* and *in vivo*; DC-derived IL-18 acts directly on T-cells to drive their conversion to Tregs. The adoptive transfer of CD4^{+}CD25^{+} T-cells from infected wild type, but not IL-18$^{-/-}$ or IL18R$^{-/-}$ animals, prevents airway inflammation and hyper-responsiveness in the above-mentioned experimental model of asthma (Oertli, 2012) (see schematic in Figure 2).

The requirement for DC-derived IL-18 in the process of *H. pylori*-specific Treg differentation is confirmed by the phenotypes of the respective gene-targeted mouse strains. IL-18$^{-/-}$ or IL18R$^{-/-}$ mice fail to develop neonatally-acquired immune tolerance to the infection, and as a consequence are significantly better able to control the infection (Oertli, 2012). A similar phenotype is seen in adult-infected IL-18$^{-/-}$ mice (Hitzler et al., 2012). We could further show that caspase-1 is activated, and IL-1b and IL-18 are processed *in vitro*

and *in vivo* as a consequence of *Helicobacter* infection (Hitzler et al., 2012). Interestingly, caspase-$1^{-/-}$ mice phenocopy IL-$18^{-/-}$ animals with respect to their hypersusceptibility to *H. pylori*-induced gastric disease. The results thus suggest an important regulatory function of caspase-1 and the inflammasome in *H. pylori* pathogenesis, which we are currently following up in more detail by screening for the responsible *H. pylori* PAMPs and identifying the pattern recognition receptors involved.

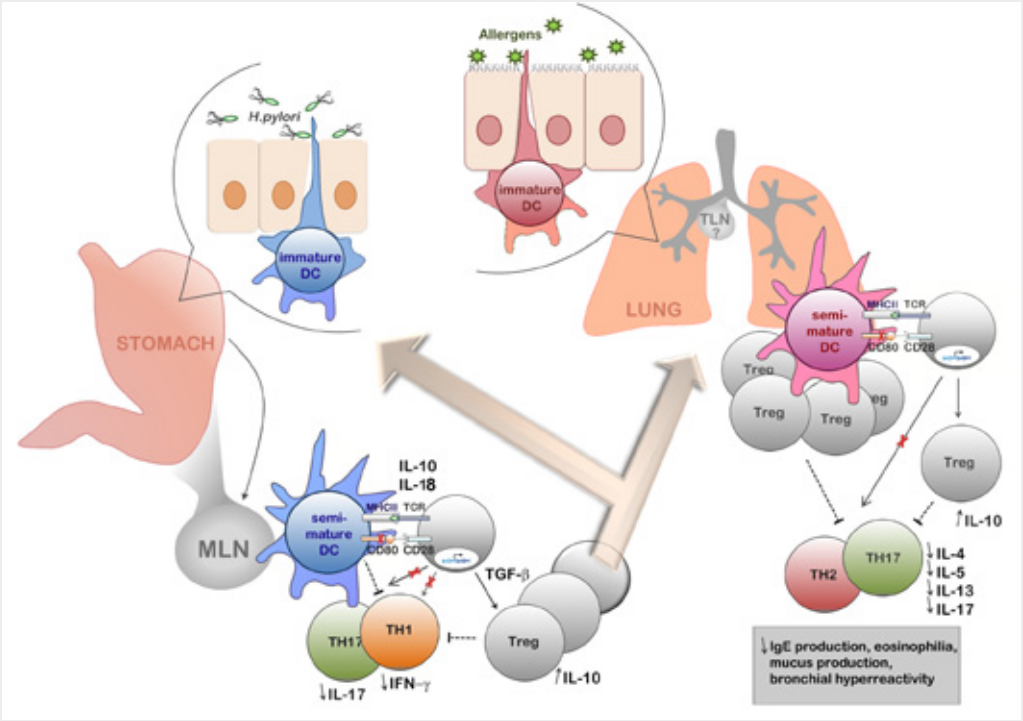

Figure 1.
Schematic representation of the current model of H. pylori-induced immune tolerance and asthma protection. Tolerogenic dendritic cells and *H. pylori*-induced regulatory T-cells act in concert to prevent adaptive Th1/Th17-driven immunity to the infection and to inhibit allergen-specific Th2 responses. In chronically infected humans, *H. pylori resides* exclusively in the gastric mucosa, where it is presumably encountered and detected by tissue-resident DC populations extending dendrites into the gastric lumen. *H. pylori*-experienced DCs migrate to the gut-draining mesenteric lymph nodes, where they act as potent inducers of TGF-b-dependent $FoxP3^{+}$ regulatory T-cells, but fail to prime *H. pylori*-specific Th1 and Th17 responses. Induced Tregs may further perpetuate the tolerogenic effects of *H. pylori*-experienced DCs by retaining mesenteric lymph node DCs in a semi-mature state and by directly suppressing *H. pylori*-specific gastric Th1 and Th17 responses, thereby protecting the host from excessive gastric immunopathology. Newly induced Tregs further migrate to the lung, where they suppress allergen-specific Th2 and Th17 responses involved in the pathogenesis of asthma. The generation of allergic T-cell responses may be blocked either through the tolerogenic effects of Tregs on DCs (retaining DCs in a semi-mature state) or directly through suppression of Th2 and Th17 responses via Treg/T-effector cell contact or via soluble cytokines, in particular IL-10. The ultimate outcome of gastric *H. pylori* infection on the allergen-challenged lung is reduced eosinophilia, mucus production and airway hyper-responsiveness. The involvement of the tracheal lymph nodes in *H. pylori*-induced asthma suppression is likely, but currently not well understood.

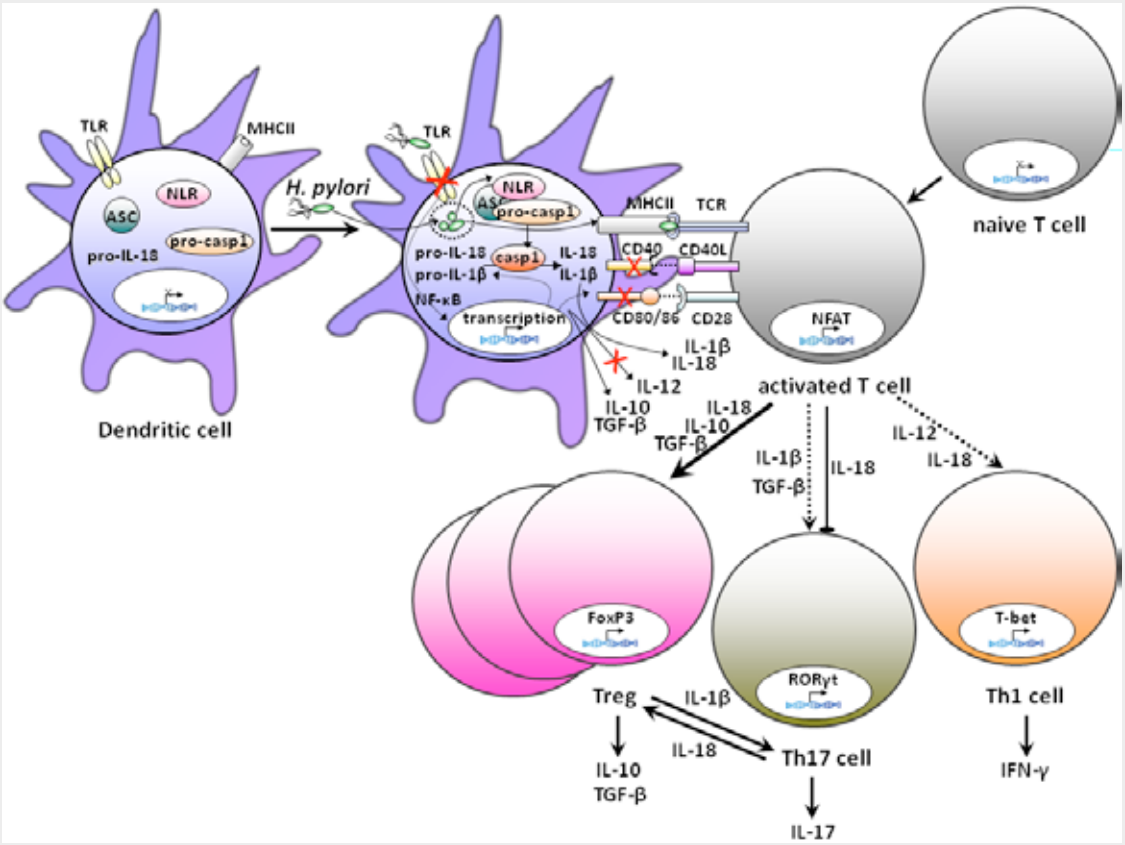

Figure 2.
Schematic representation of the effects of H. pylori exposure on DCs and the DC/T-cell interaction. Exposure to *H. pylori* induces semi-mature MHCII^{high}CD80^{lo}CD86^{lo} DCs. Inflammasome activation by *H. pylori* through as yet uncharacterized cytoplasmic Nod-like receptors (NLRs) leads to caspase-1 activation and the processing and secretion of IL-1b and IL-18. IL-1b promotes Th17 differentation, whereas IL-18 is required for Th1 and Treg differentation. *H. pylori*-experienced DCs actively induce the conversion of naive T-cells to FoxP3^{+} Tregs in a process that requires IL-18, TGF-b, and possibly IL-10. In contrast, *H. pylori*-experienced DCs are poor inducers of Th17 and Th1 differentiation. The documented lack of *H. pylori* TLR ligands in conjunction with efficient inflammasome activation by the bacteria suggests that the relative availability of pro-IL-b (low level expression due to lack of transcriptional activation) and pro-IL-18 (high levels due to constitutive expression) for caspase-1 processing may dictate the outcome of the DC/T-cell interaction.

The molecular pathogenesis of *Helicobacter pylori*-induced mucosa-associated lymphoid tissue (MALT) lymphoma

Michael Flori, Corina Schmid

In this project, we study the pathogenesis of gastric lymphoma induced by chronic infection with the gram-negative bacterial pathogen *Helicobacter pylori*. We have previously examined the specificity of the tumor immunoglobulins produced by MALT lymphoma B-cells and the role of helper T-cells in MALT lymphomagenesis. We found that the tumor immunoglobulins recognize not just one, but a multitude of unrelated foreign and self antigens - feature termed polyreactivity- (Craig et al., 2010a) and that tumor infiltration by helper T-cells is essential for primary lymphoma cell proliferation in vitro and for MALT lymphoma development in a mouse model (Craig et al., 2010b). We have further embarked on a program aimed at elucidating the contribution of small regulatory 'micro' RNAs (miRNAs) to various steps of the gastritis- low grade lymphoma- high grade lymphoma sequence of gastric lymphomagenesis. A microarray-based survey has allowed us to identify miRNA candidates with putative tumor suppressive properties.

The miRNA 203 (miR-203) was found to be specifically repressed in human low and high grade lymphomas compared to reactive gastritis samples. We determined that transcription of the miR-203 gene is specifically silenced by promoter hypermethylation in the lymphoma, but not the gastritis samples. We further found that both the forced re-expression of miR-203, as well in the pharmacological inhibition of the miR-203 target Abl, efficiently block the proliferation of primary murine MALT lymphoma cells *in vitro* and *in vivo* (Craig et al., 2011a).

Another microRNA, miR-34a, was identified in our array-based comparison of miRNA expression in low vs. high grade gastric lymphoma samples. miR-34a was found to be transcriptionally repressed in all examined cases of high grade, but not of low grade gastric lymphoma (Craig et al., 2011b). Interestingly, the forced expression of miR-34a very efficiently blocks proliferation of two high grade diffuse large B-cell lymphoma (DLBCL) cell lines, suggesting a tumor suppressive role of miR-34a in this disease entity. We have bioinformatically identified a miR-34a target with a likely functional significance in gastric lymphomagenesis, the hematopoietic oncoprotein FoxP1. Luciferase reporter assays as well as miR-34a re-expression experiments in various cell lines suggest that FoxP1 is indeed directly regulated by miR-34a at the post-transcriptional level. We plan to now investigate in a larger set of DLBCL cells lines as well as in a well-established DLBCL xenograft model whether miR-34a indeed has tumor suppressive properties -and its target FoxP1 has oncogenic properties- in MALT lymphoma. The progression from gastritis to gastric DLBCL via low grade MALT lymphoma is summarized in the schematic in Figure 3.

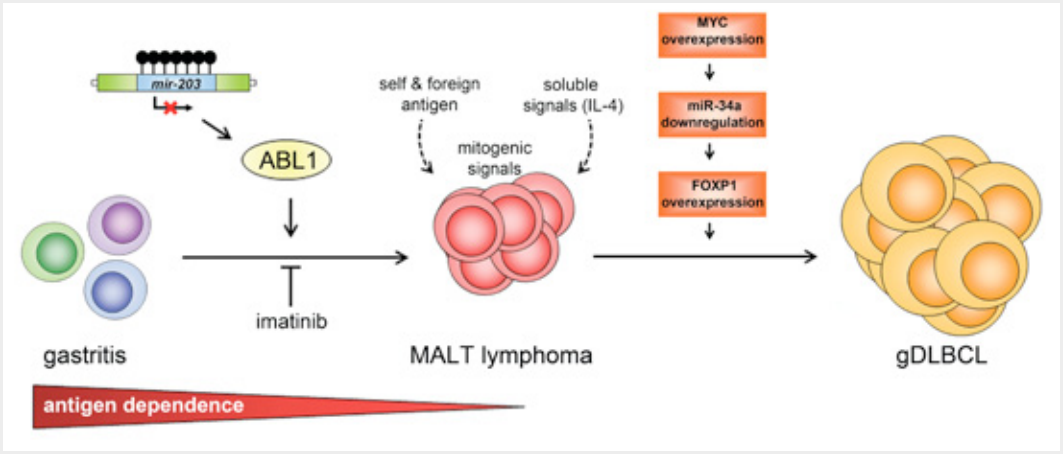

Figure 3.
Tumor cell-intrinsic and extrinsic pathways contribute to gastric lymphoma development upon *Helicobacter pylori* infection.

Helicobacter pylori triggers DNA double strand breaks and a DNA damage response in its host cells

Dorotea Gruber and Mara Hartung in collaboration with the groups of M. Lopes and A. Sartori

The molecular mechanisms of *H. pylori*-associated gastric carcinogenesis remain ill defined. In this project, we are examining the possibility that *H. pylori directly compromises* the genomic integrity of its host cells. We have recently provide evidence that the infection introduces DNA double strand breaks in primary and transformed, murine and human, epithelial and mesenchymal cells (Toller, Neelsen et al., 2011). Double strand break induction depends on the direct contact of live bacteria with mammalian cells. The infection-associated DNA damage is evident upon separation of nuclear DNA by pulse field gel electrophoresis and by high magnification microscopy of metaphase chromosomes (Figure 4). Bacterial adhesion, e.g. via the BabA adhesin, is required to induce double strand breaks; in contrast, the *H. pylori* virulence factors vacuolating cytotoxin (VacA), γ-glutamyl transpeptidase (γGT) and the Cag pathogenicity island are dispensible for DSB induction. The DNA discontinuities trigger a damage signaling and repair response involving the sequential ATM-dependent recruitment of repair factors (53BP1, MDC1) and H2AX phosphorylation. While most breaks are repaired efficiently upon termination of the infection, we observe that prolonged active infection leads to saturation of cellular repair capabilities. In summary, we conclude that DNA damage followed by potentially imprecise repair is consistent with the carcinogenic properties of *H. pylori* and with its mutagenic properties *in vitro* and *in vivo*, and may contribute to the genetic instability and frequent chromosomal aberrations that are a hallmark of gastric cancer.

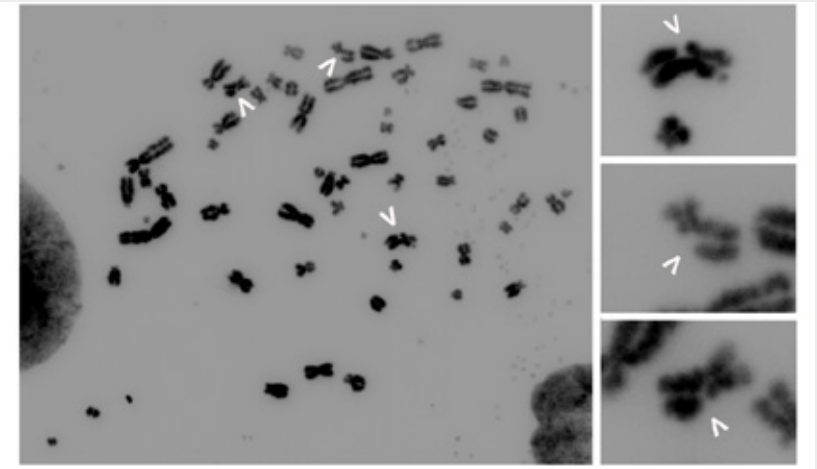

Figure 4.
Helicobacter pylori triggers DNA double strand breaks that are evident in metaphase chromosomes. Panels on the right show broken chromosomes.

Funding
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ALESSANDRO A. SARTORI

DNA DOUBLE-STRAND BREAK REPAIR AND CANCER

Studying the causes and consequences of DNA damage on the molecular and cellular level is a major topic of research within cancer biology. DNA damage not only causes cancer, but is also used as a means to cure cancer through radio- or chemotherapy. It is also responsible for the side effects of these treatments. DNA double-strand breaks (DSBs) are the most cytotoxic lesions induced by ionizing radiation and certain anti-cancer drugs, and appropriate signaling and repair of DSBs are therefore of prime importance.

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* left 2012

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 the most dangerous lesions 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.

We are deploying several approaches to achieve our aim. 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 and its regulation by post-translational modifications such as phosphorylation and ubiquitylation. DSBs are repaired by two evolutionarily conserved mechanisms: homologous recombination (HR) and non-homologous end-joining (NHEJ). However, the criteria that decide which pathway repairs which DSB remain largely unknown and elucidating how the choice is regulated is another major topic in our laboratory.

To preserve genomic integrity and aid survival, DSBs alarm the cellular DNA damage response machinery, a multifaceted process orchestrated in mammalian cells by the ATM and ATR kinase signaling pathways. In response to genotoxic insults, ATM and ATR phosphorylate key substrates involved in DNA repair and cell cycle control. In S and G2 phases of the cell cycle, DSBs are resected to produce single-stranded DNA that contribute to cell cycle checkpoint activation and trigger repair by HR. DNA end resection, a process that is still not understood in great detail, has been shown to be dependent on both ATM and CDK activities, but the targets of these kinases that are involved in DNA resection have not yet been identified.

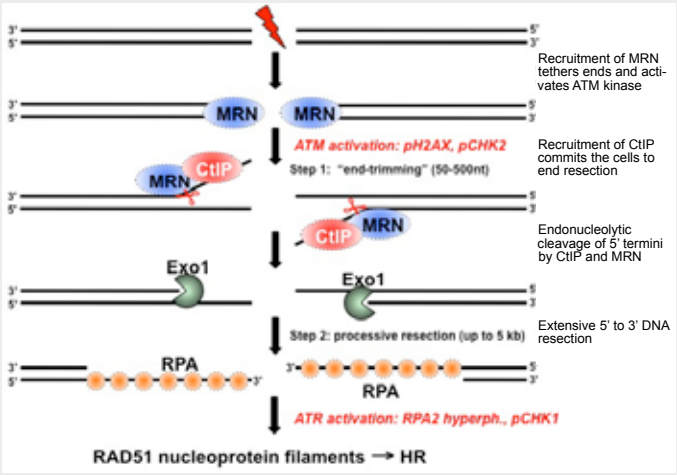

Figure 1.
CtIP is required for DNA end resection. Sensing of DSBs by MRN leads to the activation of ATM and subsequent phosphorylation of several downstream targets involved in DNA damage response (DDR) such as H2AX and CHK2. Initial DNA end resection is realized through MRN and CtIP, followed by extensive resection carried out by EXO1. The resulting 3'ssDNA overhangs are immediately coated by RPA. As a consequence, ATR is recruited and hyperphosphorylates more DDR target proteins including RPA2 and CHK1 required for G2/M checkpoint activation. Finally, RPA is exchanged for RAD51 and the RAD51-ssDNA nucleoprotein filaments initiates strand invasion and HR repair with the help of additional factors.

The identification of novel factors implicated in the DNA damage response by high-throughput screens

Martin Steger, Hella Bolck, Lorenza Ferretti, Lorenzo Lafranchi, Christine von Aesch

Human CtIP (RBBP8) was originally discovered as a cofactor of the transcriptional co-repressor CtBP. In addition to transcriptional regulation, CtIP plays a crucial role in the repair of DNA double-strand breaks (DSBs) by initiating homologous recombination. Furthermore, CtIP has been shown to interact with two tumor suppressor proteins: retinoblastoma (pRB) and BRCA1. Recent evidence suggests, that CtIP is able to counteract pRB-mediated G1 arrest while the CtIP-BRCA1 complex is important to facilitate DSB resection and subsequent repair during S and G2 phase. Genetic studies in mice revealed that CtIP is an essential gene in mammalian cells. Homozygous CtIP$^{-/-}$ mice were inviable, while haploid insufficiency predisposed mice to multiple types of tumors, indicating that CtIP might itself be a tumor suppressor. Thus, CtIP emerges as a multivalent adaptor connecting cellular pathways such as cell cycle checkpoint control, transcriptional regulation and tumor suppression, key events known to be strongly implicated in tumorigenesis and tumor progression. In order to further expand our understanding of the function(s) of CtIP in various biological pathways and to uncover new therapeutical approaches to treat cancer, we plan to conduct multiple systematic RNAi screens. Specifically, we aim to interrogate a large number of human genes for synthetic genetic interactions (synthetic lethality or rescue) with CtIP.

The regulation of DSB repair by post-translational modifications

Martin Steger, Lorenza Ferretti, Lorenzo Lafranchi, Christine von Aesch

Human CtIP is involved in the DNA damage response by promoting DNA end resection which is required for the repair of DNA double-strand breaks (DSBs) by homologous recombination (HR). Several recent studies have indicated that CtIP is under tight regulation by a number of post-translational modifications, including phosphorylation and ubiquitylation. However, while it was established that phosphorylation by CDK is required for DSB resection, it is still largely unknown how ubiquitylation and deubiquitylation controls CtIP function. For instance, it has been reported that CtIP polyubiquitylation by the BRCA1/BARD1 E3 ubiquitin ligase does not target CtIP for degradation but, instead, triggers CtIP association with chromatin following DNA damage. Besides BRCA1, a yeast-two hybrid revealed interaction of CtIP with SIAH-1, another E3 ubiquitin ligase but this study did not address whether SIAH-1 triggers CtIP ubiquitylation and subsequent degradation. It has been shown that CtIP protein levels peak in S/G2 phase while its transcript levels remain constant throughout the cell cycle, suggesting that CtIP is regulated by the ubiquitin-proteasome pathway.

In agreement with this hypothesis, we did observe a significant increase of CtIP protein levels upon treatment of cells with MG-132. Furthermore, using mass spectrometry-based proteomic screens, we have identified HECT- and RING-domain-containing E3 ubiquitin ligases as potentially novel CtIP interacting partners. In addition, we have also identified a ubiquitin hydrolase, indicative of a dynamic balance between CtIP ubiquitylation and deubiquitylation. We are now in the process of verifying these hits. Ultimately, our goal is to identify the cellular pathways promoting CtIP ubiquitylation and to understand its physiological relevance.

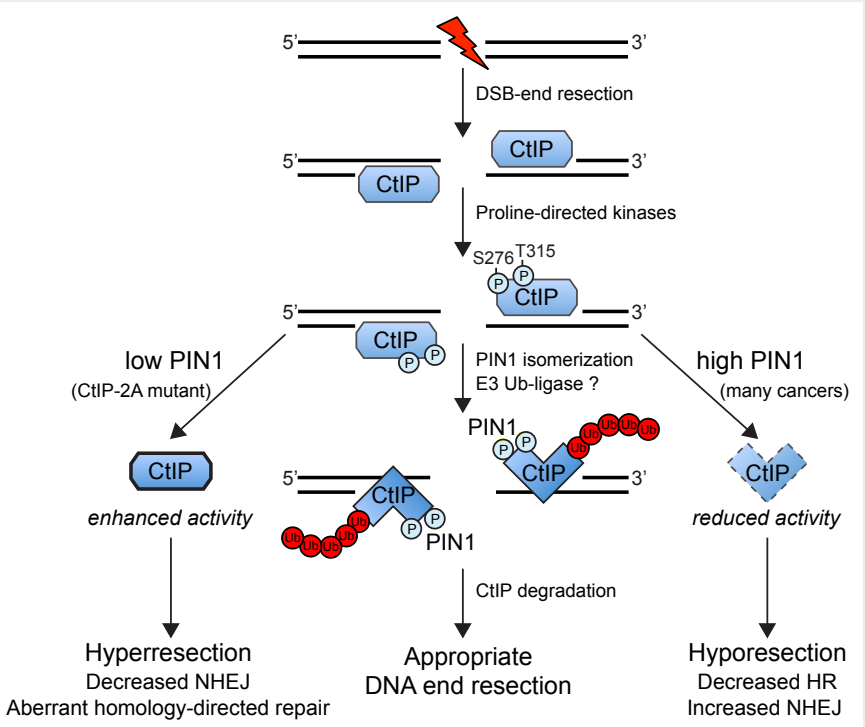

Figure 2.
Hypothetical model: how PIN1-mediated CtIP isomerization controls DNA end resection. During S/G2, CtIP together with other nucleases promotes the resection of DSBs. Following resection initiation, proline-directed kinases including CDK2 phosphorylate CtIP on T315 and S276, resulting in the binding of PIN1 to CtIP. PIN1-mediated isomerization of CtIP leads to CtIP ubiquitylation through an as yet unknown E3 ubiquitin ligase and subsequent CtIP degradation by the proteasome. This mechanism ensures an appropriate usage of DSB-end resection. Consequently, cells with abrogated PIN1 function or inherently low PIN1 levels display reduced NHEJ and aberrant (error-prone) forms of homology-directed repair due to enhanced CtIP resection activity (Hyperresection). In contrast, cells overexpressing PIN1 display reduced HR and increased NHEJ due to decreased CtIP resection activity (Hyporesection). Therefore, we propose that PIN1 plays an important role in the regulation of DSB repair particularly in late S and G2 phases of the cell cycle.

Discovering novel connections between CtIP and genome other genome surveillance pathways

Olga Murina

DNA double-strand breaks (DSBs) are one of the most critical lesions with respect to survival and preservation of genomic integrity. A key role in recognizing, signaling and repair of DSBs in mammalian cells is ascribed to the MRE11-RAD50-NBS1 (MRN) complex. Our study is aimed to gain more mechanistic insights on the role of RAD50 in the DNA damage response, which so far has been mostly attributed to serve as a scaffolding component of the MRN complex. To gain more detailed mechanistic insights into how RAD50 contributes to the maintenance of genome integrity, we are analyzing biochemical and cellular properties of human RAD50S ('Separation-of-function') mutations. More than 20 years ago, several *rad50S* alleles were isolated and characterized in *S. cerevisiae*. These alleles conferred no overt MMS sensitivity to the yeast cells but still blocked viable spore formation, indicative for a strong defect in meiosis. To reveal the underlying mechanism resulting in increased hypersensitivity of RAD50S to CPT, we are currently addressing potential repair and/or cell cycle checkpoint defects in established RAD50S-expressing human cell lines. In summary, our detailed characterization of RAD50S phenotypes using isogenic human cell lines should eventually lead to a better understanding of the function of the MRN complex in the maintenance of genome stability.

Fanconi anemia (FA) is a rare hereditary disorder characterized by bone marrow failure, multiple congenital abnormalities and increased susceptibility to cancer. Cells isolated from FA patients display chromosomal instability and hypersensitivity to DNA interstrand crosslink (ICL)-inducing agents such as mitomycin C (MMC) and cisplatin. ICLs represent highly toxic DNA lesions that prevent transcription and replication by inhibiting DNA strand separation. Recent studies indicate that FA pathway orchestrates ICL repair mediated by nucleotide excision repair (NER), translesion synthesis (TLS) and, in a final step, homologous recombination (HR). CtIP is required for normal embryonic development and promotes the resection of DSBs during HR. Thus, it is generally believed that the function of CtIP in ICL repair is through DNA end resection, downstream of the initial ICL processing step and genetically distinct from the FA pathway. Our current work is focused on the potential interplay between CtIP and the FA pathway in the repair of ICLs.

The role of CtIP in tumorigenesis and cancer

Daniela Hühn, Hella Bolck

Since its discovery more than 10 years ago as an interacting protein of CtBP, RB, and BRCA1, human CtIP has emerged as a polyvalent adaptor protein involved in the regulation of transcription and cell cycle checkpoints. Based on the partnership with these known tumorsuppressors, CtIP has been postulated to be a candidate tumor susceptibility gene itself. Support for this hypothesis came with the observation that *Ctip*$^{+/-}$ heterozygous mice develop multiple types of tumors, predominantly large B-cell lymphomas, while homozygous deletion of Ctip results in early embryonic lethality. Moreover, CtIP cooperates with MRN in the initial processing of DSBs, called DNA end resection, which is required for homologous recombination. We have provided evidence that CtIP-dependent DNA end resection may actively suppresses non-homologous end-joining (NHEJ), the second major DSB repair pathway in human cells, which simply rejoins DSB ends. Faithful repair of DSBs is crucial for the maintenance of genomic stability, as improper repair can lead to chromosomal rearrangements such as translocations. Reciprocal chromosomal translocations are implicated in the etiology of many hematologic tumors, particularly in B-cell lymphomas. The result is either the deregulation of a proto-oncogene, or the expression of a novel fusion protein with oncogenic potential. However, which DSB repair pathway gives rise to translocations and under which conditions is still an area of intensive research. In this project we would like to investigate a potential role of CtIP in the events leading to translocations and, in the first phase, to concentrate our analysis on the function of CtIP in DSB repair in Burkitt's lymphoma. Based on our findings, we hope to be able to improve predictions of the clinical outcome of current chemotherapeutic regimens in lymphomas. Moreover, data from our ongoing biochemical characterization of CtIP might reveal new avenues leading towards the development of novel therapeutic strategies in the treatment of some specific forms of lymphoma.

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Dissertations

Bregenhorn, Stephanie. Elucidating the role of mismatch repair in class switch recombination and chromatin assembly.

Mihaljevic, Boris. Role of Mismatch Repair Proteins in Response to DNA Double-strand Breaks in Human Cells.

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Sartori, Alessandro A; Steger, Martin. Prolyl isomerization: A new PIN code for DSB repair. Cell Cycle, 12(17):2717-2718.

Schmid, Corina A; Craig, Vanessa J; Müller, Anne; Flori, Michael. The role of microRNAs in the pathogenesis and treatment of hematopoietic malignancies. Current Pharmaceutical Design, 19(7):1201-1210.

Schmid, Corina; Müller, Anne. FoxD3 is a novel, epigenetically regulated tumor suppressor in gastric carcinogenesis. Gastroenterology, 144(1):22-25.

Simandlova, Jitka; Zagelbaum, Jen; Payne, Miranda J; Chu, Wai Kit; Shevelev, Igor; Hanada, Katsuhiro; Chatterjee, Sujoy; Reid, Dylan A; Liu, Ying; Janscak, Pavel; Rothenberg, Eli; Hickson, Ian D. FBH1 disrupts RAD51 filaments in vitro and modulates homologous recombination in mammalian cells. Journal of Biological Chemistry, 288(47):34168-34180.

Steger, Martin; Murina, Olga; Hühn, Daniela; Ferretti, Lorenza P; Walser, Reto; Hänggi, Kay; Lafranchi, Lorenzo; Neugebauer, Christine; Paliwal, Shreya; Janscak, Pavel; Gerrits, Bertran; Del Sal, Giannino; Zerbe, Oliver; Sartori, Alessandro A. Prolyl isomerase PIN1 regulates DNA double-strand break repair by Counteracting DNA end resection. Molecular Cell, 50(3):333-343.

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Tiwari, Amit; Schneider, Mirjam; Fiorino, Antonio; Haider, Ritva; Okoniewski, Michal J; Roschitzki, Bernd; Uzozie, Anuli; Menigatti, Mirco; Jiricny, Josef; Marra, Giancarlo. Early insights into the function of KIAA1199, a markedly overexpressed protein in human colorectal tumors. PLoS ONE, 8(7):e69473.

Dissertations

Olivera-Harris, Maite. Mismatch repair dependent processing of o6-methylguanine adducts in xenopus egg extracts.

Paliwal, Shreya. Role of RECQ5 DNA helicase in DNA double-strand break repair by homologous recombination.

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

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DNA damage response

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Mismatch repair

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Colon cancer
Colon cancer studies

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Mismatch repair

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Mismatch repair

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Liposomes
Helicobacter pilori

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Treg in MALT lymphoma

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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

Sloan Kettering Cancer Center, New York, NY, USA
DNA damage response

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 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 reapir

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

TEACHING

Cejka Petr
DNA metabolism and cancer
Genome instability and molecular cancer research
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

Janscak Pavel
DNA metabolism and cancer
Genome instability and molecular cancer research

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
Journal Club Systems Biology / Functional Genomics
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

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

Müller Anne
Animal Models of Cancerfor PhD students in the Cancer Biology program
Clinical Cancer Research
DNA metabolism and cancer
Genome instability and molecular cancer research
Scientific writing for PhD Students
Tumor immunology for PhD students in the Cancer Biology program
Vorlesung Molekulare Zellbiologie I
Vorlesung Molekulare Zellbiologie II
ETH - Infectious agents: From molecular biology to disease

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

CONFERENCES

2012

Cejka Petr
DNA repair mechanisms
Inaugural lecture, University of Zurich

Biochemical characterization of Sgs1-Top3-Rmi1: from DNA end resection to processing of entangled DNA
TU Dresden, Germany

Ferrari Stefano
Exonuclease-1 and genome stability
"Phosphorylation and Signal Transduction" in Honour of Prof. Lorenzo A. Pinna, Padova, Italy

Mechanism of 14-3-3-dependent control of DNA replication forks
Swiss Meeting on Genome Stability and Chromatin Dynamics, Weggis

Janscak Pavel
Involvement of Werner syndrome protein in MUTYH-mediated repair of oxidative DNA damage
Swiss Meeting on Genome Stability and Chromatin Dynamics, Weggis

Jiricny Josef
Non-canonical MMR
3rd Erling Seeberg Meeting, Trondheim, Norway

Eukaryotic mismatch repair: a central hub of DNA metabolism?
Anniversary Mendel Lecture, Brno, Czech Republic

Mismatch repair: tales of the unexpected
CNZ: Joint Cancer Meeting, USZ Zurich

Nucleases associated with mammalian MMR
EMBO Nuclease Workshop, Giens, France

Non-canonical MMR
FEBS 3+ Meeting, Opatija, Croatia

The mismatch repair interactome
GRC Mutagenesis, Rhode Island, USA

MMR: error free or error-prone?
IFOM, Milano, Italy

MMR: error free or error-prone?
IMB, Mainz, Germany

The mismatch repair interactome
IMP, Vienna, Austria

Genomic instability in cancer
University of Freiburg, Germany

The mismatch repair interactome
Vesalius Research Center, Leuwen, Belgium

Lopes Massimo
Structural and molecular insights into DNA replication stress
CNRS Unit of Genome Instability and Cancerogenesis, Marseille, France

DNA replication stress in cancer onset and therapy
CNZ: Joint Cancer Meeting, USZ Zurich

Structural and molecular insights into DNA replication stress
EMBO workshop on "Recombination Mechanisms and Genome Instability", Jerez de la Frontera, Spain

DNA replication stress in cancer onset and therapy
FLI-Leibniz Institute for Age Research, Jena, Germany

DNA replication stress in cancer onset and therapy
Gray Institute for Radiational Oncology, Oxford, UK

Structural and molecular insights into DNA replication stress
Institut Curie, Orsay, France

Structural and molecular insights into DNA replication stress
Institut Gustave-Roussy, Villejuif, Paris, France

Structural and molecular insights into DNA replication stress
Institute of Human Genetics, Montpellier, France

DNA replication stress in cancer onset and therapy
International Agency of Research on Cancer (IARC), Lyon, France

Uncovering DNA structures associated with DNA replication stress
Minisymposium "Image DNA damage Response", Friedrich Miescher Institute for Biomedical Research, Basel

Structural and molecular insights into DNA replication stress
Paterson Cancer Institute, Manchester, UK

Structural and Molecular Insight Into DNA Replication Stress
Swiss Meeting on Genome Stability and Chromatin Dynamics, Weggis

Structural and molecular insights into DNA replication stress
Workshop "Genome, Structure and Functions", Canceropole, Toulouse, France

Marra Giancarlo
High-throughput gene expression data during colorectal tumorigenesis: Food for systems biology research
CNZ: Joint Cancer Meeting, USZ Zurich

Gene expression dynamics in colorectal tumorigenesis
Course G1: Dynamics and maintenance of the genome, University of Basel

Müller Anne
Helicobacter pylori targets dendritic cells to induce immune tolerance, promote persistence and confer protection against asthma
10th 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
7th Biomedical Students' Symposium in Immunology and Neuroscience, University of Bonn, Germany

Pathogenic and immunomodulatory properties of Helicobacter pylori govern disease outcome in gastrointestinal and allergic disease models
Biocenter of the University of Würzburg, Germany

Helicobacter pylori targets dendritic cells to induce immune tolerance, promote persistence and confer protection against asthma
Biozentrum Basel

Helicobacter pylori targets dendritic cells to induce immune tolerance, promote persistence and confer protection against allergic asthma
Groupe d'Etudes Francais des Helicobacters, Paris, France

Impfen gegen Krebs am Beispiel Magenkrebs
Infoveranstaltung der Zürcher Krebsliga, Zurich

Systemic immunomodulation by Helicobacter pylori protects against allergic and chronic inflammatory disorders
Institut für Neuroimmunologie, Univ. of Zurich

Pathogenic and immunomodulatory properties of Helicobacter pylori govern disease outcome in gastrointestinal and allergic disease models
Institute of Medical Microbiology, Univ. of Zurich

Krebsprävention durch Impfung
Naturforschende Gesellschaft Schaffhausen

Investigating host-pathogen interactions using genome-wide approaches
Retreat of the Univ. Research Priority Program in Systems Biology, Brunnen

Systemic microRNA-34a replacement therapy for the treatment of diffuse large B-cell lymphoma
Swiss-Chinese Symposium on Cancer and Neurodegenerative Diseases, ETH Zurich

Pathogenic and immunomodulatory properties of Helicobacter pylori govern disease outcome in gastrointestinal and allergic disease models
Symposium on Infections and Cancer, DKFZ Heidelberg, Germany

DC-derived IL-18 drives Treg differentiation, murine Helicobacter pylori-specific immune tolerance, and asthma protection
World Immune Regulation Meeting, Davos

Sartori Alessandro
PIN1-mediated Isomerization of CtIP Regulates DSB Repair Pathway Choice
1st Swiss Meeting on Genome Stability and Chromatin Dynamics, Weggis

The human PIN1 isomerase regulates DSB repair pathway choice
42nd Annual Meeting of the European Environmental Mutagen Society (EEMS) Warsaw, Poland

Phosphorylation-dependent Isomerization of CtIP by PIN1 Restrains DNA End Resection
Abcam Conference on Maintenance of Genome Stability, Nassau, The Bahamas

CtIP isomerization affects DSB repair pathway choice
Invited Seminar, BRIC, Copenhagen, Denmark

2013

Cejka Petr
The nuclease activity of Dna2 restrains its vigorous DNA helicase activity
Mammalian DNA repair GRC conference, Ventura, CA, USA

Homologous recombination: the beginning and the end
University of Sussex, Genome Center, UK

Jiricny Josef
Multifaceted mismatch repair
Symposium on "Genomic instability in cancer and ageing" - SAKK, Basel

Multifaceted mismatch repair
University of Copenhagen, Denmark

Lopes Massimo
Structural and molecular insights into DNA replication stress
Abcam meeting "Chromatin, Replication and Chromosomal Stability", Copenhagen, Denmark

Remodelling of replication intermediates upon DNA replication stress
Cancer Network Zurich Retreat, Grindelwald

Replication fork reversal is a general response to replication stress in human cells
Eukaryotic DNA replication and genome maintenance meeting, Cold Spring Harbor Laboratory, USA

DNA replication stress and human disease
European Molecular Biology Laboratory, Heidelberg, Germany

Remodelling of replication intermediates upon DNA replication stress
Keystone Symposium on "DNA Replication and Recombination", Banff, Canada

Remodelling of replication intermediates upon DNA replication stress
National Institute of Health, Laboratory of Molecular Pharmacology, Bethesda, USA

Remodelling of replication intermediates upon DNA replication stress
Sussex University, Genome Damage and Stability Centre, Brighton, UK

Structural and molecular insights into DNA replication stress
University of Birmingham, School of Cancer Sciences, Birmingham, UK

Marra Giancarlo
DNA methylation in colon cancer
Institute of Rheumatology, University of Zurich

Sartori Alessandro
CDK priming phosphorylation of CtIP governs DNA-end resection in human cells
BRIC 10-year anniversary symposium on Cancer Biology, Copenhagen, Denmark

DNA double-strand break repair
Department of Rheumatology, University Hospital Zurich

CtIP phosphorylation: a paradigm for the regulation of DNA double-strand break processing
DFG Research Training Group 1739, Universität Duisburg-Essen, Germany

FANCD2-CtIP interaction primes homology-directed repair of DNA interstrand crosslink
EMBO Conference - The DNA damage response in cell physiology and disease, Cape Sounio, Greece

The human PIN1 isomerase regulates DSB repair
Keystone symposium on Genomic Instability and DNA Repair, Banff, Canada

AWARDS

Anne Müller was awarded the Götz Prize 2012.

Massimo Lopes received EMBO Young Investigator Award 2012 and the Götz Prize 2013.

SEMINARS

2012

Bianchi Marco
San Raffaele University, Italy
HMGB1 is both a Damage Associated Molecular Pattern and a cell-autonomous effector of stress response

Bétermier Mireille
Centre de Génétique Moléculaire, Paris, France
Programmed DNA double-strand breaks and their repair during developmental genome rearrangements in the ciliate Paramecium

Dianov Grigory L.
Gray Institute for Radiation, Oncology and Biology, University of Oxford, Oxford, UK
Coordination of DNA damage signaling and DNA repair

Freire Raimundo
Unidad de Investigacion, Hospital Universitario de Canarias, Tenerife-Spain
Different regulations during S phase: Wee1 controlling DNA replication and control of Claspin by ubiquitin

Heinis Christian
Ecole Polytechnique Federal de Lausanne (EPFL)
Generation of bicyclic peptides binding with high affinity and specificity to targets of interest. Case study: development of a uPA antagonist to inhibit tumour growth

Hülsken Jörg
Dr. Ernst Th. Jucker Prize winner, EPFL, Lausanne
Tumor stroma interactions in metastasis establishment

Lakin Nick
University of Oxford, UK
DSB repair in Dictyostelium

Lusser Alexandra
Innsbruck Medical University, Austria
Chromatin regulation by the ATP-dependent chromatin remodeling factor CHD1

Lüscher Bernhard
Medical School, Institute of Biochemistry and Molecular Biology, Aachen University, Germany
Intracellular mono-ADP-ribosylation, a new partner in signaling and the control of cell physiology

Mailand Niels
University of Copenhagen, Denmark
New functions of ubiquitin in cellular responses to replication stress

Mirkin Sergei M.
White Family Chair in Biology Tufts University Medford, MA, USA
Explosive force of simple DNA repeats

Nicolas Alain
Institut Curie Paris, France
Role of G-quadruplexes on genetic instability

Pichierri Pietro
Istituto Superiore di Sanità, Rome, Italy
Functional interplay between the RecQ helicase WRN and the DNA replication checkpoint in human cells

Rogler Gerhard
Division of Gastroenterology & Hepatology, USZ
The function of selected Crohn's disease risk genes in mucosal immunology

Rothstein Rodney
Institute for Cancer, Columbia University, USA
Exploring the DNA damage response using high throughput screens and cell biology

Sorensen Claus
Biotech Research and Innovation Centre, Copenhagen, Denmark
Coordination between DNA damage checkpoint and repair pathways

Wang Zhao-Qi
Leibniz Institute for Age Research - Fritz Lipmann Institute Jena, Germany
Novel regulation of the S phase checkpoint by homeostasis of poly(ADP-ribosyl)ation

Wolfrum Jürgen
Institute of Physical Chemistry, University of Heidelberg, Germany
BioQuant - a new Center for Quantitative Analysis of Molecular and Cellular Biosystems at Heidelberg University

Woodgate Roger
Laboratory of Genomic Integrity, National Institutes of Health, Bethesda, Maryland, US
Ribonucleotide incorporation by E.coli DNA polV and its effects on genome instability

2013

Altmeyer Matthias
Department of Disease Biology, University of Copenhagen, Denmark
Chromatin modifications in response to DNA damage: a balancing act

Blanco Luis
Centro de Biología Molecular Severo Ochoa, CSIC-UAM, Madrid, Spain
Specialized DNA polymerases repairing double strand-breaks and initiating DNA synthesis

Burma Sandeep
Department of Radiation Oncology, University of Texas Southwestern Medical Center, USA
Targeting DNA double-strand break repair in glioblastoma

Hammond Ester
Gray Institute for Radiation Oncology and Biology - Department of Oncology, University of Oxford, UK
Hypoxia activation of the DNA damage response

Hofmann Thomas G.
DKFZ, Heidelberg, Germany
Mechanisms of DNA damage- induced cell death signaling

Jacobs Heinz
The Netherlands Cancer Institute, Amsterdam, The Netherlands
Molecular parameters and origin of chromosomal translocations in mature B cells

Khanna Kum Kum
Signal Transduction Laboratory, Queensland Institute of Medical Research Brisbane, Australia
DNA damage repair: from genome maintenance to novel therapeutic approaches for triple-negative breast cancer

Klungland Arne
Centre for Molecular Biology and Neuroscience, Oslo University Hospital, Rikshospitalet, Norway
Lost in hydroxylation; Alkbh mediated genome repair and regulation

Krejci Lumir
National Centre for Biomolecular Research and Department of Biology, Masaryk University, Brno, Czech Republic
Keeping recombination at bay

Liberali Prisca
Institute of Molecular Life Sciences, UZH
Mapping Functional Genetic Interactions From Parallel Phenotypic Screens

Meshorer Eran
The Hebrew University of Jerusalem, Israel
A library of endogenously labeled fusion proteins in embryonic stem cell reveals a novel histone chaperone involved in pluripotency and differentiation

Natoli Gioacchino
European Institute of Oncology (IEO), Milano, Italy
Understanding inflammation through genomics

Polo Sophie
Epigenetics and Cell Fate Centre, Université Paris Diderot, Paris, France
Chromatin bookmarking for transcription restart after DNA damage

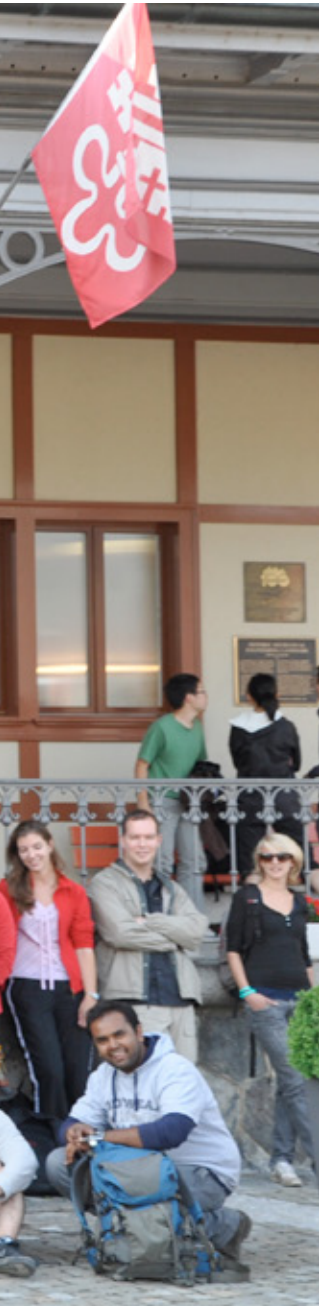
Porro Antonio
EPFL Lausanne
DNA damage response at dysfunctional telomeres: new actors for an old DDRama

Reik Wolf
Cambridge Cancer Centre, UK
Epigenetic reprogramming in mammalian development

van den Brook Maries
Institute of Experimental Immunology, UZH
The interaction between cancer and the immune system

van Vugt Marcel
University of Groningen, the Netherlands
Cytogenetic profiling of >16,000 cancers uncovers essential genes for the survival of genomically unstable tumors

Wolski Stefanie
Rudolf Virchow Centre, Würzburg, Germany
Structural and Functional Characterization of a Nucleotide Excision Repair Protein



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