SCIENTIFIC REPORT

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Institute of Molecular Cancer Research

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FOREWORD

The Institute of Molecular Cancer Research (IMCR) is continuing to enjoy its stay on the Irchel Campus of the University. Its ties to the neighbouring institutes, specifically the Institute of Veterinary Biochemistry and Molecular Biology (IVBMB) housed on the floor above, and the Institute of Molecular Biology (IMB) in the neighbouring building have become even stronger. IMCR also continues to profit from the ready access to the numerous facilities and technology platforms of the University, such as the Functional Genomic Center Zurich (www.fgcz.uzh.ch) and the Center for Microscopy and Image Analysis (www.zmb.uzh.ch).

In August of 2011, we were rejoined by Petr Cejka, an alumnus, who has returned to us from his extended postdoctoral stay at the University of California in Davis. Petr obtained the prestigious Bursary Assistant Professorship of the Swiss National Science Foundation and is in the process of building up his first independent research group.

As in previous years, the Institute said good-bye to several PhD students and postdoctoral research assistants who completed their training and moved onto pastures new. In compensation, we were able to attract an even larger number of new talent from all over the world, primarily through the PhD Program in Cancer Biology, which has grown into the third largest PhD Program of the Life Science Zurich Graduate School (www.lszgs.ch). This program recruits top students not only into the IMCR, but also into the research groups of the Cancer Network Zurich (www.cnz.uzh.ch) and other units of the University, the University Hospital and the ETH.

With the arrival of the Cejka group and the new student intake, the Institute has now reached its full capacity. It houses nearly 80 persons, 13 of whom (3 senior scientists, 3 administrative and 7 technical staff) are financed by the University. The remainder of the positions are filled by PhD students and postdoctoral research assistants, all financed by third party funds.

I look forward to another period of exciting and innovative research. My long-term goal has been to position IMCR among the leading institutes of molecular cancer research. The young and talented team we have on board at the present time should make this goal not only easier to achieve, but will undoubtedly make it also fun. It is a pleasure and a privilege to be at the helm of such an exciting venture.

JOSEF JIRICNY

Director

IMCR CREW

SCIENTIFIC ADVISORY BOARD

Susan Gasser (Friedrich Miescher Institute, Basel, Switzerland)

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

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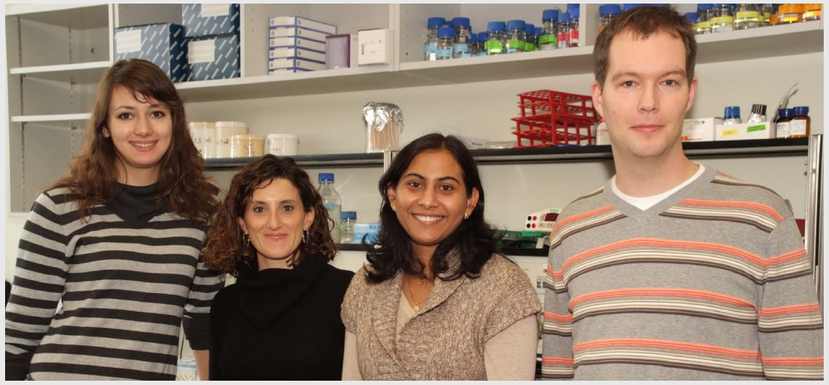

Repair of damaged DNA by homologous recombination and related pathways

Elda Cannavó, Lepakshi, Maryna Levikova

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.

Specifically, the goal of our laboratory is to understand detailed molecular mechanisms of homologous recombination. Double strand DNA break repair is initiated by the nucleolytic (degradative) processing of one of the DNA strand near the break (so-called DNA end resection). It is now clear that DNA ends are processed by several nucleolytic complexes. We are investigating the detailed reaction mechanisms, as well as how the processing is affected by the presence of various chemical groups attached to DNA in the vicinity of the breaks. DNA end-resection is essential for the subsequent assembly of key recombination proteins that search for homologous DNA. Following the exchange of homologous DNA strands and the formation of joint molecules, a number of recombination factors then ensure proper separation and resolution of these joint structures. We are also investigating the processing of recombination intermediates by various enzyme complexes. Finally, homologous recombination is not only essential to repair double strand DNA breaks; the complex can also address single-strand DNA breaks (or gaps in one of the DNA strands), and it may help restart stalled/collapsed replication forks. However, these important functions of homologous recombination are mechanistically more complicated than double strand DNA break repair and are thus much less understood. We are investigating the functions of several protein complexes that link stalled replication machinery with homologous recombination.

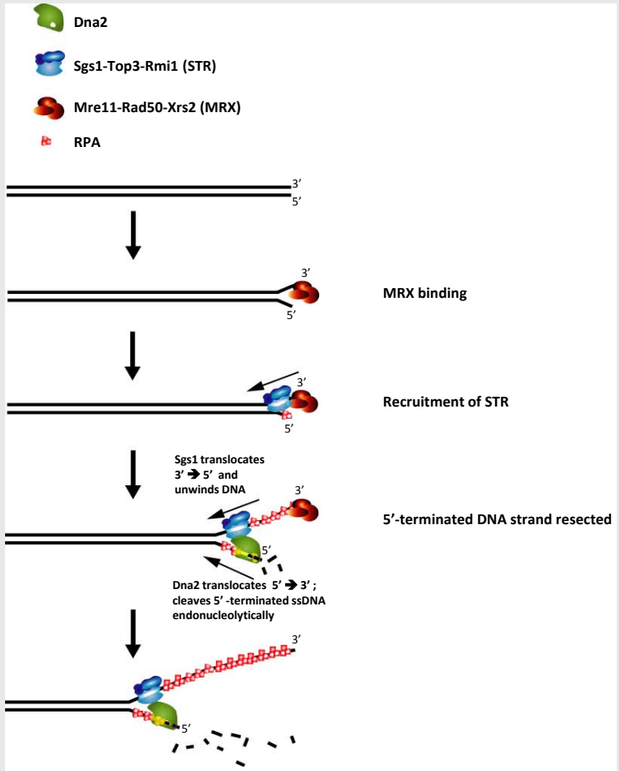

Figure 1.
Model for resection of a DNA double-strand break by Dna2, Sgs1, Top3, Rmi1, Mre11, Rad50, Xrs2, and RPA.

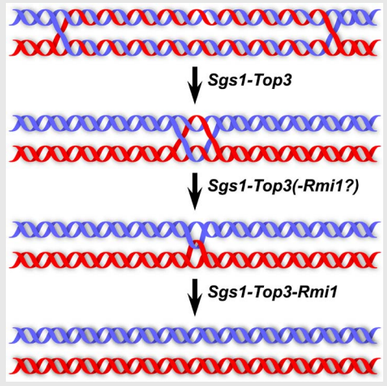

Figure 2.
Model of Sgs1–Top3–Rmi1 complex function in the dissolution of double Holliday junctions.

Funding
Swiss National Science Foundation.

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. General objective of our work is to elucidate the wiring of checkpoint pathways and the regulation of repair mechanisms put in place to address DNA damage. Rationale for this approach is the attainment of a proof-of-principle on the suitability of checkpoint pathways as targets in cancer therapy.

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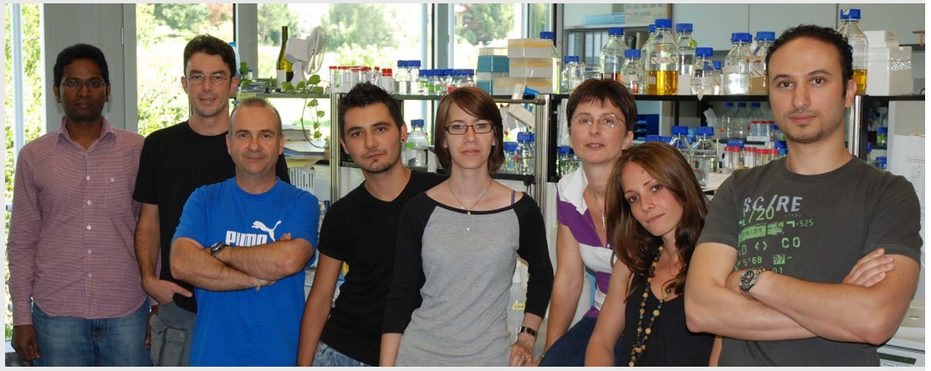
* left 2011



Exonuclease 1 and DSB repair

Wassim Eid

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 be recruited to stalled replication forks, where it counteracts fork reversal.
The focus of our work is on pathways and molecular mechanisms that control EXO1 at DSBs and stalled DNA replication forks. We have previously shown that EXO1 is a low abundant protein, that is constantly expressed throughout the cell cycle and that undergoes rapid degradation in response to stalled DNA replication (El-Shemerly et al., 2005). Evidence obtained using selective inhibitors, as well as by monitoring post-translational modifications, revealed that EXO1 protein stability is regulated through phosphorylation-dependent poly-ubiquitylation, followed by 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 as well as of ATR-dependent pathways and we identified 12 sites of phosphorylation in EXO1 (El-Shemerly et al., 2008).

DNA end resection, which is essential for the repair of DSBs by homologous recombination, is initiated by MRE11-RAD50-NBS1 (MRN) and CtIP. This step is followed by processive resection involving helicases and exonucleases such as EXO1.
We have obtained evidence that the localization of EXO1 to DSBs depends on both CtIP and MRN. However, we observed that EXO1 does not interact with the MRN complex, but that it directly binds CtIP. The latter not only recruits EXO1 to DSBs, but also modulates its exonucleolytic activity. Finally, we observed that in cells exposed to the topoisomerase-I inhibitor camptothecin, depletion of EXO1 and CtIP led to an increase in the frequency of chromosomal rearrangements, among which the formation of radial chromosomes was a prominent feature. We demonstrated that gross chromosomal rearrangements were the result of illegitimate repair of DSBs by NHEJ (Eid et al., 2010).
By showing that CtIP and EXO1 act in concert to protect cells from the deleterious consequences of end-joining-mediated repair, our study uncovered novel important functions for these two proteins and shed new light on the regulation of DSB repair pathways and on their role in the maintenance of genome integrity.

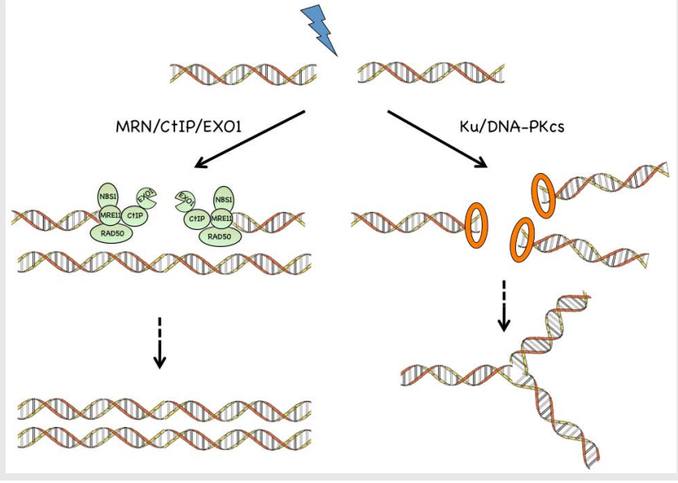


Figure 1.
Model of DSB repair. Binding of MRN and CtIP to DNA ends promotes initial trimming and is followed by CtIP-dependent recruitment of EXO1. The latter carries out processive resection to generate intermediates that are suitable for recombination (left). This avoids the formation of gross chromosomal rearrangements that may result from illegitimate NHEJ-dependent repair of DSBs (right).

Exonuclease 1 and DNA replication

Kim Engels, Murat Aykut, Chappidi Naga Raja

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. Exonuclease 1 (EXO1) was previously implicated in pathological processing of stalled replication forks and other sensitive DNA intermediates in checkpoint-defective yeast cells. By means of a two-hybrid screen, we identified 14-3-3 proteins as in vivo interaction partners of human EXO1 and were able to confirm that such interaction occurs also between Bmh1/Bmh2 (yeast 14-3-3 proteins) and yeast Exo1 (Engels et al., 2011).
14-3-3 proteins are able to bind phosphorylated proteins and were shown to play an undefined role under DNA replication stress.
We found that 14-3-3-deficient yeast cells failed to induce Mec1-dependent Exo1 hyperphosphorylation and, as a consequence, accumulated Exo1-dependent ssDNA gaps at stalled forks, as revealed by electron microscopy. This, in turn, led to persistent checkpoint activation and exacerbated recovery defects. Interestingly, deletion of *EXO1* in 14-3-3-deficient cells completely suppressed this phenotype, leading to a reduction of the ssDNA gaps behind the fork to a level similar to wild type cells, but did not affect fork progression (Engels et al., 2011). This indicates that 14-3-3 proteins are required to prevent unscheduled Exo1 activity behind stalled replication forks and control additional unknown targets to promote fork stability and restart in response to DNA replication stress.

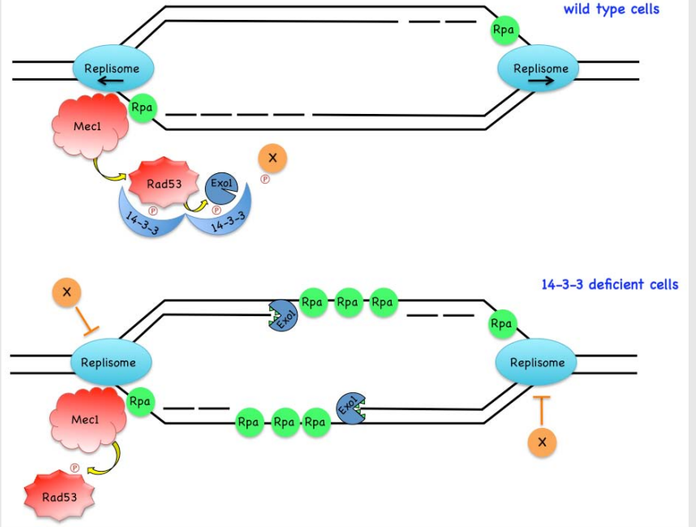


Figure 2.
Model of 14-3-3-mediated control of replication. The 14-3-3 proteins likely function as a platform to facilitate Mec1/Rad53-dependent phosphorylation of Exo1 and other unknown targets (X), contributing to replication fork stabilization (top). In 14-3-3 deficient cells, lack of control of 14-3-3 targets causes the formation of ssDNA gaps behind the fork, persistent checkpoint activation and exacerbated recovery defects, which gives rise to genomic instability.

Control of human Exonuclease 1 stability

Serena Bologna

As mentioned above, while yeast Exo1 is controlled by phospho-dependent protein-protein interactions, human EXO1 undergoes phospho-dependent ubiquitylation and degradation in response to stalled replication forks.
In order to identify the pathway controlling EXO1 protein stability under conditions of replication stress, we are screening an E2 ubiquitin-conjugating enzyme siRNA library in mammalian cells that express GFP-EXO1. The extent of green fluorescence and the level of GFP protein are used as read-out in immunofluorescence and Western blot experiments, respectively, to evaluate and quantify the stability of EXO1 upon down-regulation of each E2. Next, E3 ubiquitin ligases that are established partners of the identified E2s will be examined using the assays described above. Finally, the pathway of EXO1 ubiquitylation will be reconstituted *in vitro* and its role on genome stability will be verified in cellular models.

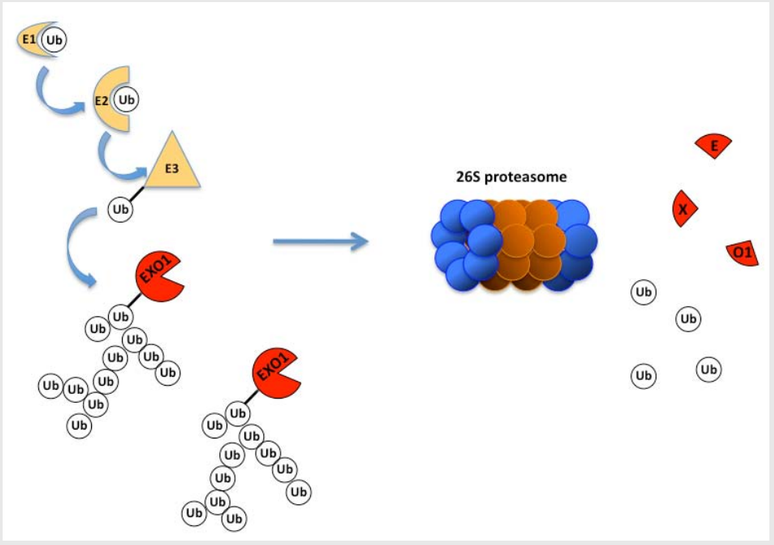


Figure 3.
Pathway of human EXO1 degradation in response to replication stress.

Funding
Huggenberger-Bischoff Stiftung, URPP, Promedica Stiftung, Stiftung Wissenschaftliche Forschung UZH, Stiftung für Krebsbekämpfung, SNF/ProDoc Program.

PAVEL JANSCAK

BIOCHEMISTRY OF DNA REPAIR

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. Research in our laboratory focuses on understanding various DNA repair mechanisms operative in mammalian cells. Our main aim is to define the exact DNA transactions mediated by RecQ DNA helicases, key players in the maintenance of genomic stability.

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Boris Mihaljevic
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Judith Schroeder

* left 2011

Repair of oxidative DNA damage

Radhakrishnan Kanagaraj, Boris Mihaljevic
(in collaboration with Prof. Ulrich Hübscher)

Reactive oxygen species constantly generated as by-products of cellular metabolism readily attack genomic DNA generating a variety of oxidized DNA bases that have a high mutagenic potential. One of the most frequent oxidative DNA lesions is 7,8-dihydro-8-oxo-guanine (8-oxo-G). These lesions accumulate in the genome with age and are believed to contribute to the aging process and to the development of various types of cancer. All known replicative DNA polymerases show a strong tendency to misincorporate dATP opposite 8-oxo-G, which results in G:C to T:A transversions. 8-oxo-G:A mispairs are primarily eliminated by the base excision repair (BER) system. Repair is initiated by the MutY DNA glycosylase homologue (MUTYH), which removes the mispaired A residue in a long-patch BER reaction wherein DNA polymerase λ (Polλ) incorporates dCTP opposite 8-oxo-G. The resulting 8-oxo-G:C pair is processed by the OGG1 glycosylase in a short patch BER reaction leading to the removal of 8-oxo-G and restoration of the initial G:C pair. We have investigated whether the repair of 8-oxo-G:A mispairs requires the WRN helicase/exonuclease as its germ-line defects cause a premature aging condition termed Werner syndrome, and lack of WRN in cells leads to accumulation of oxidative guanine lesions and accelerated telomere attrition. Using DNA-protein cross-linking in mammalian cell extracts, we found that WRN was specifically recruited to a DNA duplex containing an 8-oxo-G:A mispair in a manner dependent on Polλ. Similarly, by immunofluorescence, we observed that Polλ was required for the recruitment of WRN to sites of 8-oxo-G lesions in human cells. We also found that nuclear focus formation of WRN and Polλ induced by oxidative stress was dependent on ongoing DNA replication and on the presence of MUTYH, and that depletion of MUTYH conferred oxidative stress tolerance to cells lacking WRN and/or Polλ. Biochemical studies with purified proteins demonstrated that WRN interacted directly with the catalytic domain of Polλ and specifically stimulated DNA gap filling by Polλ across 8-oxo-G. Together, these results suggest that WRN promotes the bypass of 8-oxo-G lesions by Polλ during MUTYH-initiated repair of 8-oxo-G:A mispairs. It is therefore possible that the accelerated telomere shortening observed in cells derived from Werner syndrome patients stems from persistence of 8-oxo-G lesions within the telomeric G-strand due to defective repair of 8-oxo-G:A mispairs.

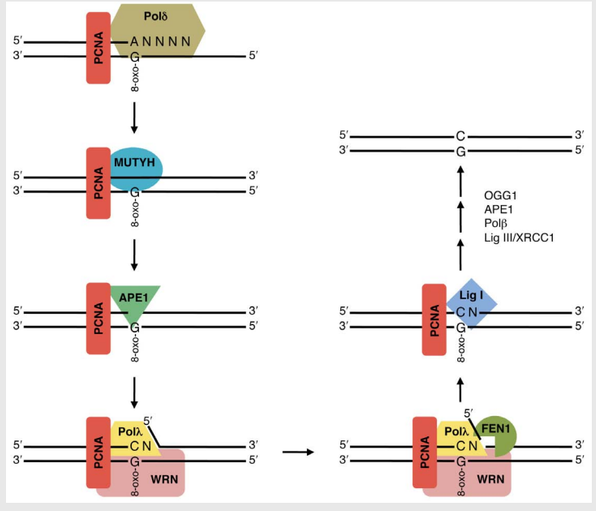

Figure 1.
A model for the role of Werner syndrome protein (WRN) in MUTYH-initiated repair of 8-oxo-G:A mispairs arising during DNA replication. WRN stimulates long-patch DNA synthesis by DNA polymerase λ (Polλ) across the 8-oxo-G lesion. WRN might also coordinate the removal of 5′-flap by FEN1. The resulting 8-oxo-G:C intermediate is processed by short-patch BER initiated by the OGG1 glycosylase.

Regulation of homologous recombination

Shreya Paliwal, Boris Mihaljevic

Homologous recombination (HR) mediates accurate repair of DNA double-strand breaks (DSBs) arising during S/G2 phases of the cell cycle where the undamaged sister chromatid can serve as a repair template. HR is initiated by nuclease-mediated resection of a DSB to generate 3′-single-stranded (ss) DNA tails that are coated by the ssDNA-binding protein RPA. In the next step, the RAD51 recombinase binds to these ssDNA tails with help of BRCA2 to form a nucleoprotein filament that catalyzes the invasion of the donor sister chromatid, giving rise to a three-stranded structure called a displacement (D)-loop. After DNA synthesis primed by the invading strand, the repair can bifurcate into two alternative sub-pathways referred to as classical DSB repair (DSBR) and synthesis-dependent strand annealing (SDSA). In DSBR, the second DSB end is captured to form an intermediate with two Holliday junctions, called a double Holliday junction (DHJ). This DNA structure can be resolved by specialized endonucleases into crossover or non-crossover products, or dissolved by a concerted action of the BLM helicase and DNA topoisomerase IIIα in a complex with an OB-fold containing protein RMI1 (the BTR complex), giving rise exclusively to non-crossover products. In SDSA, the extended D-loop is disrupted by a specialized DNA helicase such as RTEL1, and the newly synthesized DNA is annealed to the ssDNA tail of the other break end, which is followed by gap-filling DNA synthesis and ligation. SDSA always results in non-crossover products and appears to be the preferred HR pathway during mitosis in mammalian cells. However, the underlying molecular mechanism is poorly understood.
Our research focuses on the role of RECQ5 DNA helicase in the regulation of HR in human cells. In collaboration with the laboratories of Dr. Patrick Sung and Dr. Guangbin Luo, we have established that RECQ5 suppresses HR events leading to crossover products and that it has the ability to disrupt RAD51 nucleoprotein filaments in vitro. Our current studies using chromosomally-based reporters for different HR pathways in combination with biochemical approaches address the possibility that RECQ5 promotes SDSA by disrupting aberrant RAD51 filaments formed on newly synthesized DNA following D-loop disruption.

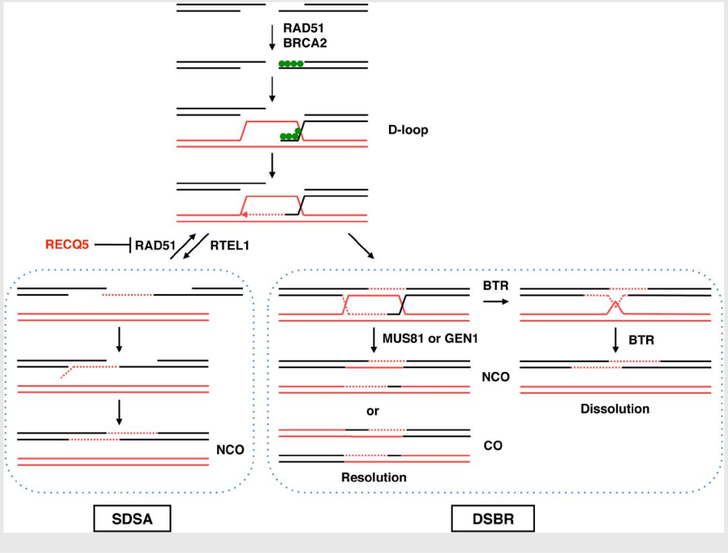

Figure 2.
Homologous recombination pathways. The main models for repair of DNA double-strand breaks by homologous recombination (HR) are shown. RECQ5 is proposed to promote SDSA by disrupting aberrant RAD51 filaments that might form on the newly synthesized DNA after unwinding the extended D-loop by RTEL1. BRCA2 promotes the assembly of RAD51 filament at the initiation stage of HR and hence counteracts the filament disruption activity of RECQ5.

Resolution of conflicts between transcription and replication machineries

Radhakrishnan Kanagaraj, Daniel Hühn, Judith Schroeder

Transcription can induce DNA recombination by causing a collapse of replication forks. However, there exist mechanisms in the cell that promote genomic stability upon collision between transcription and replication complexes, which are yet not well understood. Our studies in human cells have shown that during the productive elongation phase of transcription, the catalytic subunit of RNA polymerase II, Rpb1, associates with the RECQ5 DNA helicase through a conserved motif, termed Set2-Rpb1-interacting domain, that specifically binds to the Ser2/Ser5-phosphorylated form of the C-terminal heptapeptide repeat domain of Rpb1. Other studies have shown that depletion of RECQ5 from human cells results in formation of DNA double-strand breaks during S-phase in a manner dependent on transcription. Our current studies aim to address the exact role of RECQ5 in preventing transcription-associated genomic instability.

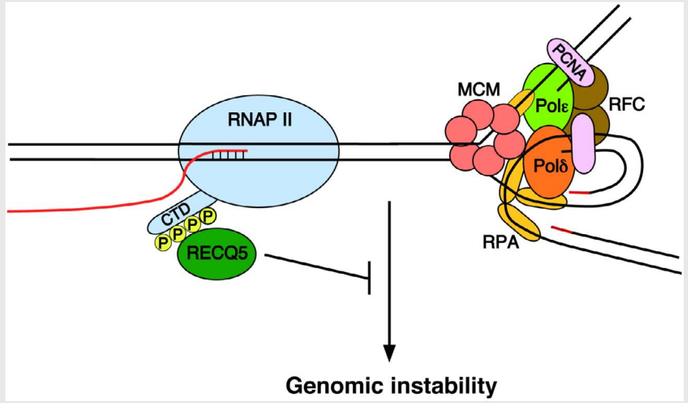

Figure 3.
A model for the role of RECQ5 during transcription. Head-on collision of RNA polymerase II (RNAPII) complex with replisome can lead to fork breakage and genomic instability. RECQ5 associates with the C-terminal domain (CTD) of RNAPII during the elongation phase of transcription by means of a conserved CTD-binding motif that specifically recognizes CTD heptapeptide repeats phosphorylated at Ser2 and Ser5. We hypothesize that RECQ5 is involved in the mechanisms controlling genomic stability upon collision between transcription and replication.

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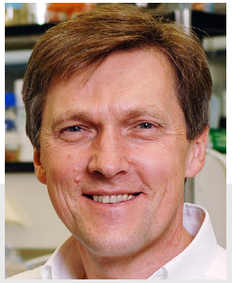
JOSEF JIRICNY



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), 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 focussing on some of these processes. Much of the work in the laboratory is focussed on the biochemistry of human MMR proteins, but we have also began exploring 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, Kalpana Surendranath

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. During the course of this study, several components of the MMR pathway could be shown to be post-translationally modified. Although some of these modifications are constitutive, others are clearly inducible. Given that the minimal reconstituted system does not display quite the same substrate specificity as the cell-free systems or as MMR *in vivo*, we postulate that these differences may be due to post-translational modifications of the participating proteins, and/or that some non-essential components of the MMR pathways remain to be identified.

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. Phenotypic analysis of these cells revealed that all three cell lines had substantially elevated mutation frequencies (Figure 1), which implies either that their MMR capacity was saturated, or that MMR does not address errors that escape the proofreading exonuclease.

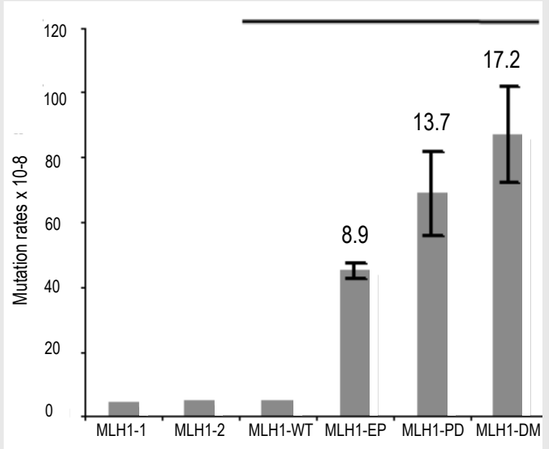

Figure 1.
Elevated mutation frequency in cells expressing variants of polymerase-δ. Mutation frequencies measured at the *HPRT* locus of human MMR-proficient A2780 clone MLH1-1 and -2 cells, in which endogenous polymerase-δ was knocked down by shRNA and in which the wild type (WT) polymerase-δ, or its error-prone (EP), proofreading-deficient (PD) or the double mutant (DM) were simultaneously expressed from a stably-integrated cDNA vector.

MMR and interstrand cross-link repair

Svenja Kaden, Katja Kratz, Barbara Schöpf

We identified KIAA1018 as a strong interactor of the MMR protein MLH1. Using DT40 cells, we were able to show that cells lacking this protein were hypersensitive to cisplatin and other agents that induce interstrand cross-links (ICLs). As sensitivity to ICL-inducing agents is a hallmark of *Fanconi anemia*, we set out to characterise KIAA1018 in greater detail. We could show that KIAA1018 is an exo/endonuclease, which preferentially cleaves 5′ flaps and D-loops *in vitro* (Figure 2A). As anticipated, knock-down of KIAA1018 *in vivo* resulted in sensitivity of the cells to cisplatin and mitomycin C, but it also lowered recombination efficiency and double-strand break repair. We therefore postulate that KIAA1018 is involved in the processing of recombination intermediates, such as D-loops, *in vivo*. Because KIAA1018 could be shown to be recruited to chromatin by mono-ubiquitylated *Fanconi* protein FANCD2 (Figure 2B), it has been renamed FANCD2-associated nuclease 1, FAN1. We are now trying to understand the biological relevance and/or importance of the binding of FAN1 to MLH1 and PMS2.

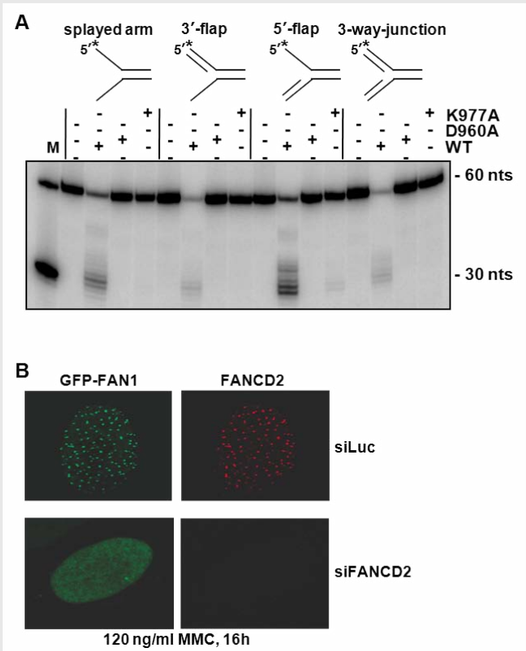

Figure 2.
Characterization of KIAA1018/FAN1. (A) The purified recombinant FAN1 protein was incubated with the indicated 60-mer substrates labelled in the strand indicated by an asterisk. As shown, the enzyme displays preference for 5′ flap structures. (B) Localization of GFP-FAN1 to mitomycin C-induced DNA damage foci is dependent on FANCD2 (upper panel). In cells in which the latter protein was knocked down by siRNA (lower panel), GFP-FAN1 is diffused throughout the nucleus.

The role of MMR proteins in antibody diversification

Stephanie Bregenhorn, Javier Peña-Diaz

The generation of our vast antibody repertoire involves three processes, in which the genome of B cells is irreversibly altered: VDJ recombination, somatic hypermutation (SHM) and class switch recombination (CSR). 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 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 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, during SHM/CSR, MMR proteins appear to act as mutators. Using defined uracil-containing substrates, we could show that base excision repair and MMR interfere with one another during the processing of substrates containing more than one uracil, such as would arise after the processive action of AID (Figure 3). This interference gives rise to long repair tracts, which trigger mono-ubiquitylation of PCNA that has been reported to be necessary for the recruitment to DNA of translesion polymerases such as polymerase-η. Given that the latter enzymes are error-prone, we postulate that the deployment of translesion polymerases in the filling of MMR-generated gaps leads to mutations during SHM/CSR.
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.

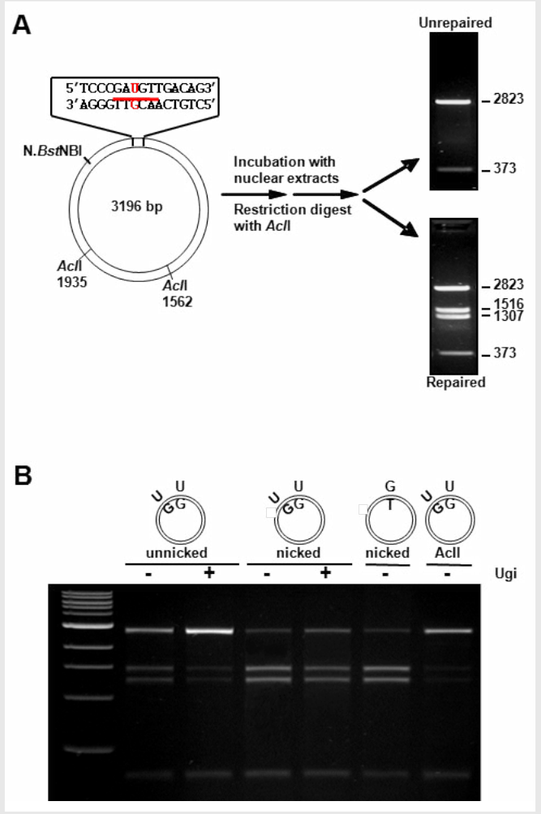

Figure 3.
Interference of MMR and BER during the processing of uracil-containing substrates. (A) Schematic representation of the in vitro mismatch repair assay. The presence of the G/T mismatch in the *Acl*I restriction site makes the plasmid refractory to cleavage by the enzyme. Digestion with this enzyme thus gives rise to two fragments, of 2823 and 373 base pairs. G/T to A/T repair in human nuclear extracts restores the third *Acl*I site, which gives rise to two additional fragments of 1516 and 1307. The intensity of the bands is indicative of repair efficiency. (B) A G/T mismatch in the *Acl*I site (red) is repaired with high efficiency, providing the substrate contains a nick in the outer strand (lane 3-5). Similar repair efficiency is seen also in an unnicked substrate containing a uracil residue 50 nucleotides 5′ from the mispaired G (lane 1), but this repair can be inhibited by the addition of uracil glycosylase inhibitor (Ugi) to the extracts (lane 2). This indicates that an intermediate of uracil processing by BER was used by the MMR system as an initiation site. Lane 6. Control showing that the G/T substrate is resistant to *Acl*I digestion. M, marker.

Repair of modified nucleotides

Milica Enoiu, Maite Olivera

The MMR system has been shown to be involved also in the processing of DNA damage other than base/base mismatches and IDLs. It appears to be responsible for the cytotoxicity of the mutgenic 6-*O*-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 the resistance phenomenon, 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 "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 that uses extracts of human cells is based on the correction of the mispair in an nicked heteroduplex substrate. This does not faithfully mirror the process in which a mispair is addressed immediately after it was generated by the polymerase. 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.

Funding
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MMR and chromatin assembly

Barbara Schöpf and Stephanie Bregenhorn (in collaboration with Geneviéve Almouzni and Jean-Pierre Quivy, Institut Curie)

We could show that the human mismatch-binding factor MutSα interacts with the large subunit of the heterotrimeric chromatin-assembly factor (CAF1) *via* the N-terminus of MSH6. This interaction appears to inhibit chromatin assembly in an *in vitro* assay, in which a mismatch-containing plasmid is actively repaired by the MMR system. Given that MutSα needs to slide on the DNA in order to interact with its downstream partners and activate MMR, nucleosomes would hinder this function and thus prevent efficient mismatch correction. We postulate that the CAF1-MSH6 interaction has evolved to delay the reloading of nucleosomes onto newly-replicated DNA that contains mismatches, which would facilitate the repair process.

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, affecting various aspects of human disease, such as 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 fibers/combing, 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

DNA replication interference is one of the most common strategies employed in the clinics 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 1), 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. This important set of data has recently been published in *Nature Structural and Molecular Biology*. We are now committed to test the contribution of specific cellular factors, likely to play a role in 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 revert 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 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.

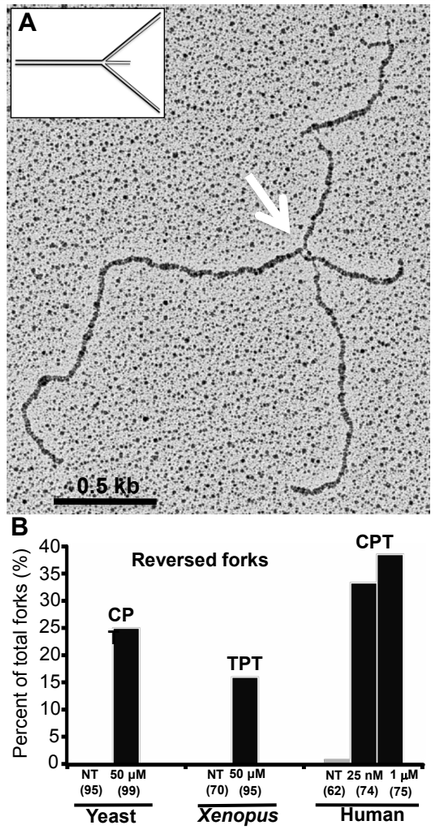

Figure 1.
Top1 poisoning results in replication fork reversal. (A) Representative electron micrograph and schematic drawing of a reversed fork observed on genomic DNA from CPT-treated U2OS cells. The white arrow points to the four-way junction at the replication fork. (B) Frequency of fork reversal in 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, Isabella Zanini

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

The replication stress phenotype can be reproduced in cell culture by overexpression of various oncogenes influencing DNA replication, e.g. *Cyclin E*, *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, most notably reversed forks, upon induction of Cdc25A and CycE. However, only the overexpression of Cdc25A, and not of CycE, causes massive DNA breakage and full DDR activation shortly after oncogene induction (Figure 2). We found that Cdc25A-dependent DNA double strand breaks (DSB) are suppressed by preventing mitotic events (chromosome condensation, Holliday junction resolution). Intriguingly, suppression of HJ resolution is associated with accumulation of reversed replication forks, suggesting that, at least upon Cdc25A overexpression, these unusual replication intermediates can be processed into DSB. We therefore propose that oncogene-induced replication stress promotes replication fork reversal and that oncogene-dependent DSB are due to premature activation of mitotic factors. We are currently testing the contribution in these phenomena of cellular factors suspected to induce or process reversed forks. Moreover, we are exploiting a system recently established in the lab to investigate re-replication, a pathological phenomenon frequently associated with oncogene activation. Finally, we will extend our studies to more oncogenes and to their effect on primary cells.

Structural analysis of DNA replication across unstable repetitive sequences

Cindy Follonier, Judith Oehler

A growing number of human neurological hereditary diseases - among which Huntington disease, Freidreich's Ataxia and fragile-X 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 clarified that TNR can pause DNA replication fork progression. 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 indeed 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 insight 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. By large-scale molecular biological approaches, we also recently isolated these abnormal DNA structures and used them to generate specific antibodies, in collaboration with the specialized group of Dr. Mori in Japan. We succeeded in isolating a promising structure-specific antibody and we are currently testing it for *in vivo* immuno-fluorescence and several *in vitro* assays. We aim to use this antibody to generate a simple read-out for genomic instability arising at replicating chromosomes.

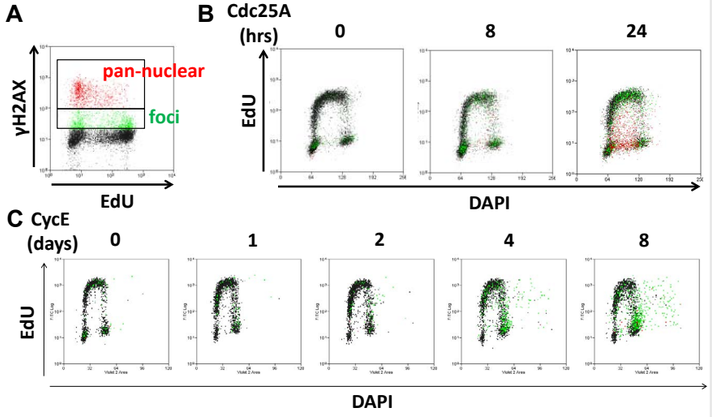

Figure 2.
Flow cytometric analysis of DNA synthesis, cell cycle progression and DDR activation after oncogene expression. (A) FACS-based distinction of γH2AX patterns after Cdc25A induction. Red and green signals indicate cells with pan-nuclear γH2AX and γH2AX foci, respectively. (B) FACS analysis after 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.

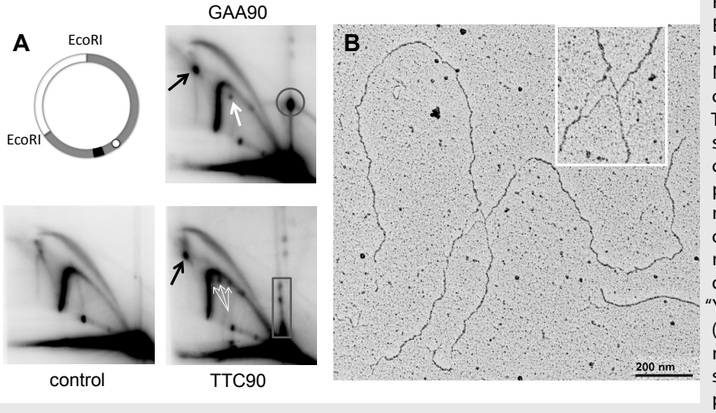

Figure 3.
Expanded GAA/TTC repeats induce unusual replication intermediates in human cells. (A) Neutral-neutral 2D-gel analysis of plasmids containing the indicated numbers of GAA or TTC repeats as template for lagging strand synthesis. Plasmids were transfected in 293T cells, recovered after 40h, digested by EcoRI, 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 (Figure 3A, black arrow) from GAA90 plasmid EcoRI-fragment. Magnification 46kx.

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 genome during active proliferation. We recently started to investigate the intriguing connection between replication stress and aging, applying some of our most revealing approaches to different populations of stem cells (ESCs and HSCs). We have found that ESCs exhibit high levels of the endogenous DNA damage marker γH2AX, which markedly decreases 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 in basal 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 on 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.

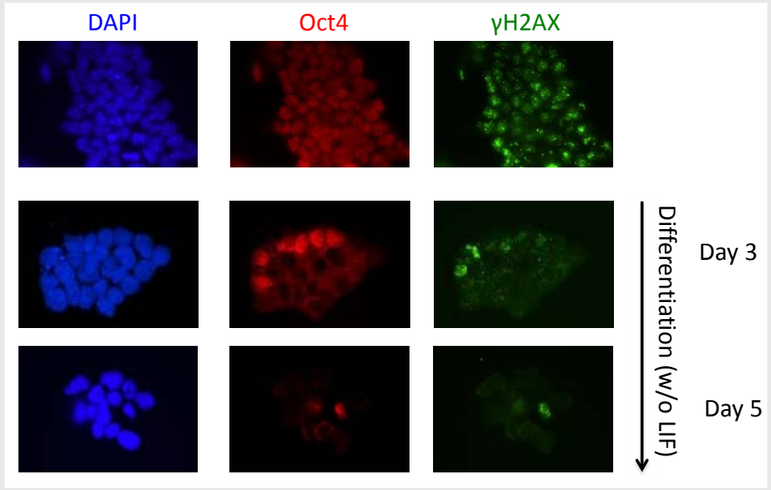

Figure 4.
Kinetics of ESC differentiation reveals that γH2AX is lost upon the onset of differentiation. ESCs accumulate high levels of γH2AX, which are rapidly lost upon induction of differentiation. γH2AX is lost in parallel to the stem cell marker Oct4 at an early stage of differentiation, when the cells are still undergoing active proliferation (data not shown).

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Swiss National Science Foundation, URPP Systems Biology/ Functional Genomics, Krebsliga Zurich, Fonds zur Förderung des akademischen Nachwuchses.

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 2010

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 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.

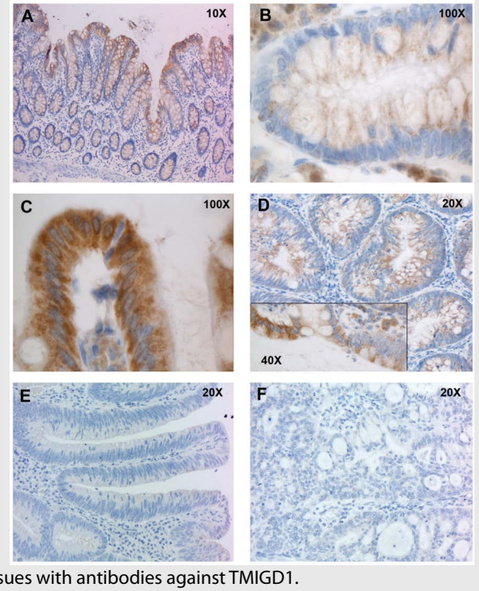

Figure 1. Immunohistochemical staining of normal and neoplastic colonic tissues with antibodies against TMIGD1.
(A) In normal mucosa, TMIGD1 expression is limited to the upper portion of the epithelial crypts, where differentiated cells are located. (B) Higher magnification views of TMIGD1 staining at base of a colonic crypt. (C) Higher magnification views of TMIGD1 staining at mouth of a colonic crypt. TMIGD1 is located in the cytoplasm and probably in the cell membrane. (D) Its expression was markedly reduced in nonpolypoid lesions. The inset shows different levels of expression at the interface between normal (left) and dysplastic (right) epithelium. (E) More marked reduction was observed in polypoid lesions. (F) expression was lost in colorectal cancers.

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.

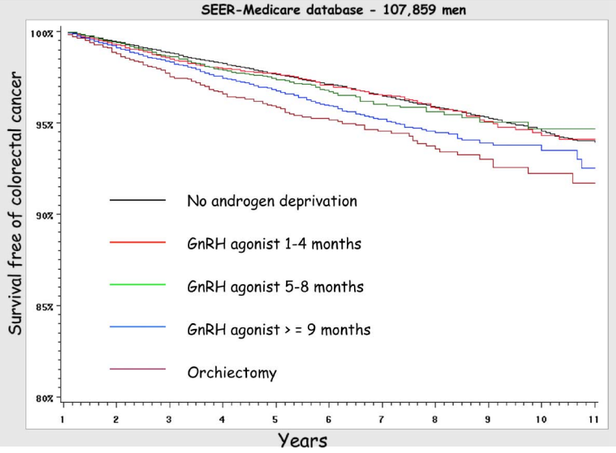

Figure 2.
Kaplan-Meier curves of colorectal cancer-free survival starting at 12 months following diagnosis of prostate cancer for patients undergoing androgen deprivation initiated within one year of diagnosis. The cohort was subdivided into orchiectomy, GnRH agonist use 1-4, 5-8, ≥ 9 months in the first 12 months after diagnosis, versus those who received no androgen deprivation in the first 12 months after diagnosis.

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.

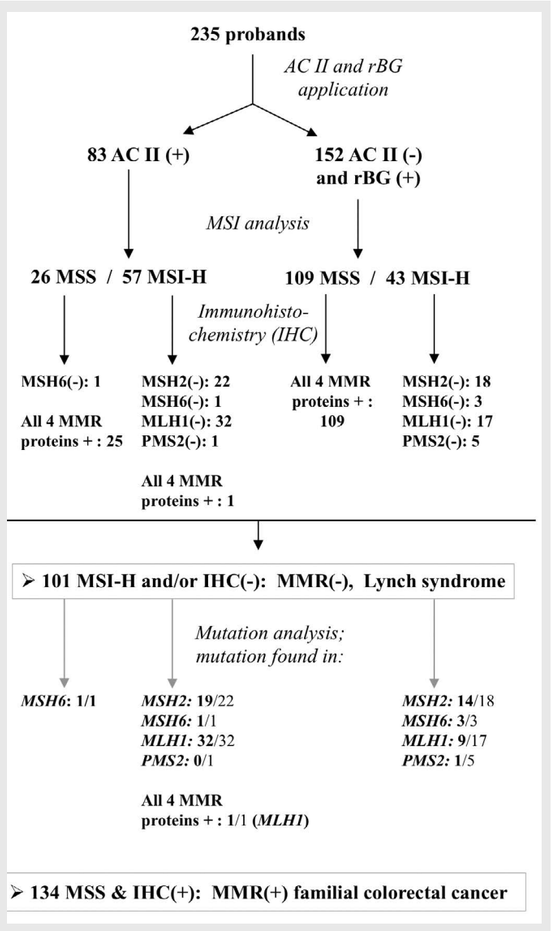

Figure 3. This figure summarizes the results of our analysis of 235 kindreds with nonpolyposis forms of familial colon cancer. Eighty three (35%) families fulfilled the AC II (Amsterdam criteria II); the remaining 152 (65%) satisfied one or more of the inclusion criteria of the rBG (revised Bethesda guidelines). On the basis of the results of MSI (microsatellite instability) analysis and IHC (immunohistochemistry) of tumor samples from the probands, the familial colon cancer in 101 of these 235 kindreds (43%) was classified as MMR (mismatch repair)-deficient. (In 79 of these cases, mutation screening of germ-line DNA revealed a deleterious variation in the unexpressed MMR gene, thereby confirming the diagnosis of Lynch syndrome.) The other 134 (57%) were MMR-proficient.

Analysis of secretome changes uncovers an autocrine/paracrine component in the modulation of cell proliferation and motility by c-Myc

G. Pocsfalvi, G. Votta, A. De Vincenzo, I. Fiume, D.A. Raj, G. Marra, M.P. Stoppelli, I. laccarino

Proteins secreted by cancer cells are a major component of tumor microenvironment. However, little is known about the impact of single oncogenic lesions on the expression of secreted proteins at early stages of tumor development. Because c-Myc overexpression is among the most frequent alterations in cancer, we investigated the effect of sustained c-Myc expression on the secretome of a nontransformed human epithelial cell line (hT-RPE). By using a quantitative proteomic approach, we identified 125 proteins in conditioned media of hT-RPE/MycER cells upon c-Myc induction. Analysis of the 49 proteins significantly down-regulated by c-Myc revealed a marked enrichment of factors associated with growth inhibition and cellular senescence. Accordingly, media conditioned by hT-RPE cells expressing c-Myc showed an increased ability to sustain hT-RPE cellular proliferation/viability. We also found a marked down-regulation of several structural and regulatory components of the extracellular matrix (ECM), which correlates with an increased chemotactic potency of the conditioned media toward fibroblasts, a major cellular component of tumor stroma. In accordance with these data, the expression of the majority of the genes encoding proteins down-regulated in hT-RPE was significantly reduced also in colorectal adenomatous polyps, early tumors in which c-Myc is invariably overexpressed. These findings help to elucidate the significance of c-Myc overexpression at early stages of tumor development and uncover a remarkable autocrine/paracrine component in the ability of c-Myc to stimulate proliferation, sustain tumor maintenance, and modulate cell migration.

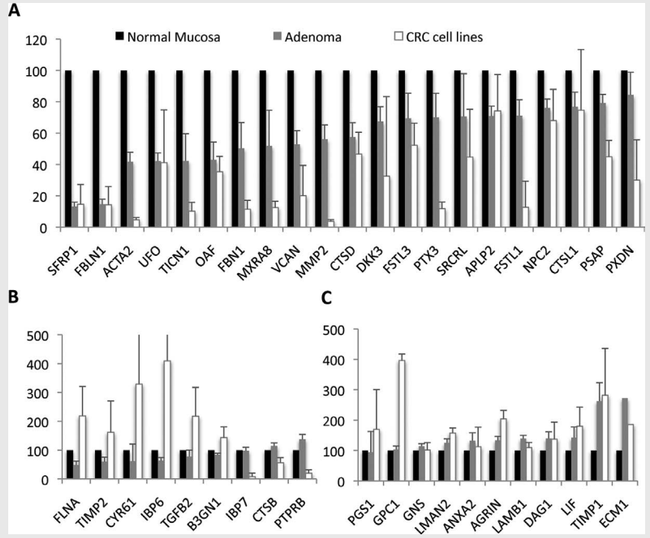

Figure 4.
Secreted factors down-regulated by c-Myc are also down-regulated in colorectal adenomas and colorectal cancer (CRC) cell lines. The expression of the genes coding for the proteins down-regulated by c-Myc activation in conditioned media of hT-RPE/MycER cells was analyzed in microarray data sets (Affymetrix U133 Plus 2.0 platform) from 34 normal colon mucosa samples, 34 colon adenomas and 18 CRC cell lines. Data are shown as % of the mean expression values in normal mucosa samples. (A) Genes down-regulated in both adenomas and in CRC cell lines; (B) genes down-regulated either in adenomas and or in CRC cell lines; (C) genes up-regulated in both adenomas and in cell lines.

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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, Isabelle Arnold, Daniela Engler

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 reprogram 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 re-

spect 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.

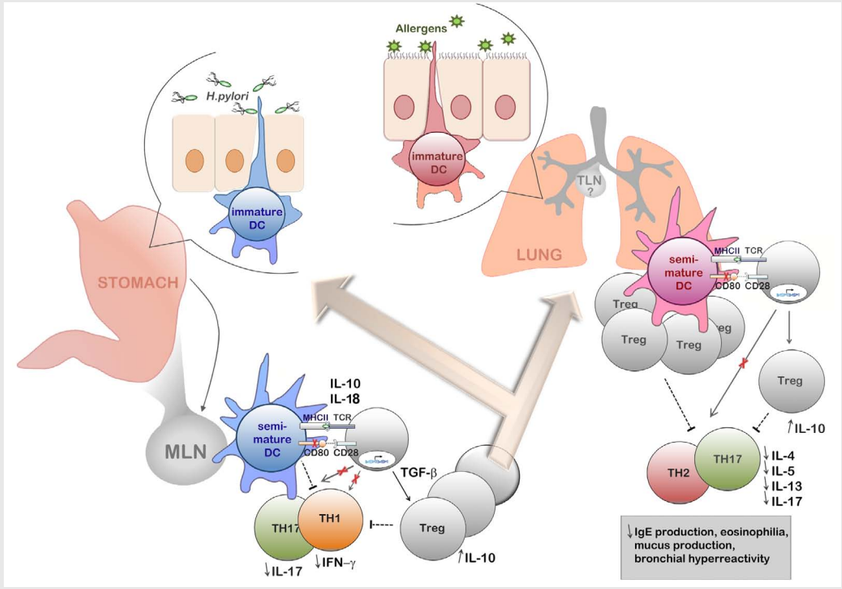

Figure 1.
Schematic representation of the current model of *H. pylori*-induced immune tolerance and asthma protection. Tolerogenic dendritic cells and *H. pylori*-induced regulatory T-cells act in concert to prevent adaptive Th1/Th17-driven immunity to the infection and to inhibit allergen-specific Th2 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.

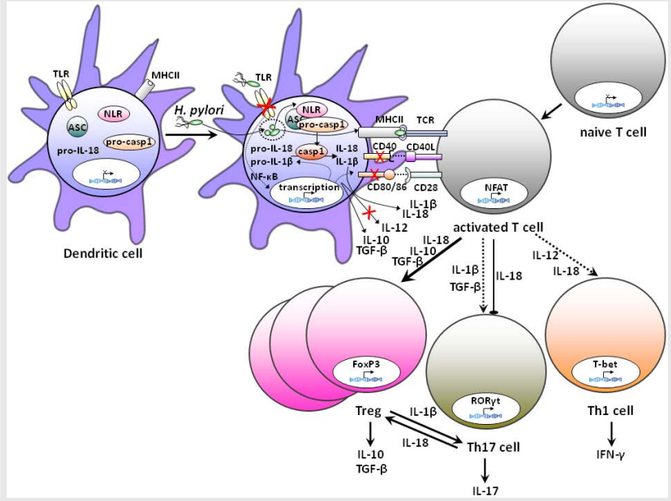

Figure 2.
Schematic representation of the effects of *H. pylori* exposure on DCs and the DC/T-cell interaction. Exposure to *H. pylori* induces semi-mature MHCII-^{high}CD80^{lo}CD86^{lo} DCs. Inflammasome activation by *H. pylori* through as yet uncharacterized cytoplasmic Nod-like receptors (NLRs) 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.

Characterization of *H. pylori*-specific effector and regulatory lymphocyte populations, and their role in controlling *Helicobacter*-induced gastric preneoplastic pathology

Mathias Oertli, Isabelle Arnold, Daniela Engler, Ayca Sayi, Iris Hitzler

Observational studies in human carriers have long supported the view that precancerous lesions in the *H. pylori*-infected stomach are immunopathological in origin, i.e. that they arise as a consequence of T-cell-driven adaptive immune responses to the infection. To experimentally address the role of *H. pylori*-specific effector and regulatory T-cells and their signature cytokines in *Helicobacter* control and the associated gastric (immuno-) pathology, we have used an extensive collection of >30 mouse strains with targeted deletions of various cytokine and lymphocyte-specific genes in two complementary models of *Helicobacter* (*pylori* and *felis*) infection. Mice lacking a T-lymphocyte compartment (Rag-1$^{-/-}$, TCR-b$^{-/-}$) were found to be incapable of controlling *Helicobacter* infections and, as a consequence, are completely resistant against infection-associated gastritis and gastric preneoplasia; the adoptive transfer of CD4^{+} effector T-cells from infected wild type donors to infected Rag-1$^{-/-}$ or TCR-b$^{-/-}$ recipients is sufficient to restore the full range of preneoplastic pathology (Arnold, 2011b; Arnold, 2011c; Sayi, 2009; Sayi, 2011). The pathogenic functions of CD4^{+} effector T-cells require the T-cell-intrinsic production of IFN-g and are under the strict control of regulatory lymphocyte populations (Sayi, 2011). The type IV secretion system substrate and suspected bacterial oncoprotein CagA is of particular interest in this context, as CagA-specific T-cells were as pathogenic as polyclonal *H. pylori*-specific T-cells in the adoptive transfer model (Arnold, 2011b). We could show using a combination of bioinformatic and experimental techniques that the central and C-terminal domains of CagA harbor several MHCII-restricted T-cell epitopes, which elicit functional T-cell responses *in vivo* (Arnold, 2011b). The *H. pylori*-specific activation of effector T-cells further requires the T-cell-intrinsic expression of a small regulatory RNA, miR-155 (Oertli, 2011). miR-155$^{-/-}$ mice fail to control *H. pylori* infection as a result of impaired pathogen-specific Th1 and Th17 responses, are less well protected against challenge infection after *H. pylori*-specific vaccination than their wild-type counterparts, and develop less severe infection-induced immunopathology (Oertli, 2011).
A substantial part of our efforts in the past years has centered on understanding in detail the role of Tregs and other regulatory lym-

phocyte populations in *H. pylori* pathogenesis on the one hand, and other disease manifestations on the other. Tregs are induced very efficiently in the course of a *Helicobacter* infection; in fact, it appears as if *H. pylori* has evolved to *preferentially* induce T-regulatory over T-effector responses to ensure its persistence in the host (Oertli, 2012). The role of Tregs is discussed in detail below; here, I would like to point out that B-cells, as well as T-cells, have regulatory properties in the context of *H. pylori* infections. We could show in a recent paper that B cells activated by *Helicobacter* TLR-2 ligands induce IL-10–producing $CD4^+CD25^+$ T regulatory-1 (Tr-1) cells *in vitro* and *in vivo* (Sayi, 2011) (see schematic in Figure 3). Tr-1 differentation depends on TCR signaling and a direct T-/B-interaction through CD40/CD40L and CD80/CD28. B cell-induced Tr-1 cells have suppressive activity *in vitro* and suppress *Helicobacter*-associated immunopathology *in vivo*. One of the more unexpected findings of this study was that the B-cell/Tr-1-mediated immunosuppression depended on T cellular, but not B cellular, IL-10 production (shown using cell-type-specific IL-$10^{-/-}$ mice) (Sayi, 2011).

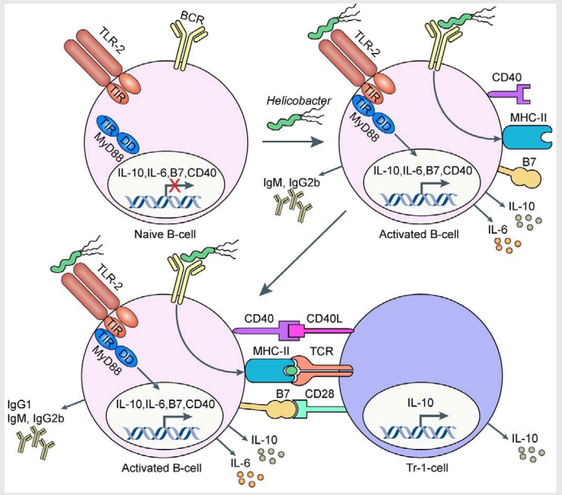

Figure 3.
Schematic representation of the events occurring in the course of *Helicobacter*-specific activation of B-cells at the site of infection. *Helicobacter* TLR-2 ligands activate B-cells in a MyD88-dependent manner, which leads to the expression and surface exposure of CD80, CD86, and CD40, and the secretion of IL-10, IL-6 and TNF-a as well as antibodies of the IgM and IgG2b subclasses. The interaction of activated B-cells and naive T-cells induces T-cellular IL-10 expression and suppressive activity in a manner dependent on a direct interaction between both cell types via CD40/CD40L, B7/CD28 and MHCII/TCR. IL-10 secreting T-cells are essential players in the prevention of *Helicobacter*-associated immunopathology.

Prevention of gastric cancer through the development of a *Helicobacter pylori*-specific vaccine

Iris Hitzler

Immunization against the gastric bacterium *Helicobacter pylori* is projected to prevent a significant fraction of gastric cancers and other gastric disorders attributed to this pathogen. Most vaccination protocols currently used in preclinical models are not suitable for humans. New adjuvants and a better understanding of the correlates and requirements for vaccine-induced protection are needed to accelerate vaccine development to *H. pylori*. We have assessed vaccine-induced protection against *H. pylori* infection and its local and systemic immunological correlates in cholera toxin- and CAF01-adjuvanted vaccination models. The contribution of B-cells, CD4+ Th-cell subsets and dendritic cells to *H. pylori*-specific protection were analyzed in appropriate mouse strains. Parenteral administration of a whole cell sonicate combined with the mycobacterial cell wall-derived CAF01 adjuvant induces protection against *H. pylori* that depends on cell-mediated, but not humoral immunity. The vaccine-induced control of *H. pylori* is accompanied by mixed Th1/Th17 responses in the gastric mucosa and in the gut-draining mesenteric lymph nodes; both Th subsets are absolutely required for *H. pylori*-specific protective immunity. The gastric numbers of memory CD4+ T cells and neutrophils were identified as the best correlates of protection induced by both vaccines. The systemic depletion of dendritic cells or of regulatory T-cells during challenge infection significantly enhances protection by overcoming immunological tolerance mechanisms, which are activated by live *H. pylori* and suppress protective immunity. In conclusion, the parenteral immunization with a novel, mycobacterially adjuvanted vaccine induces cell-mediated, Th1/Th17-polarized immunity to *H. pylori*. Tolerance mechanisms mediated by dendritic cells and regulatory T-cells impair *H. pylori* clearance and must be overcome for improved immunity.

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

Vanessa Craig, 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 4.

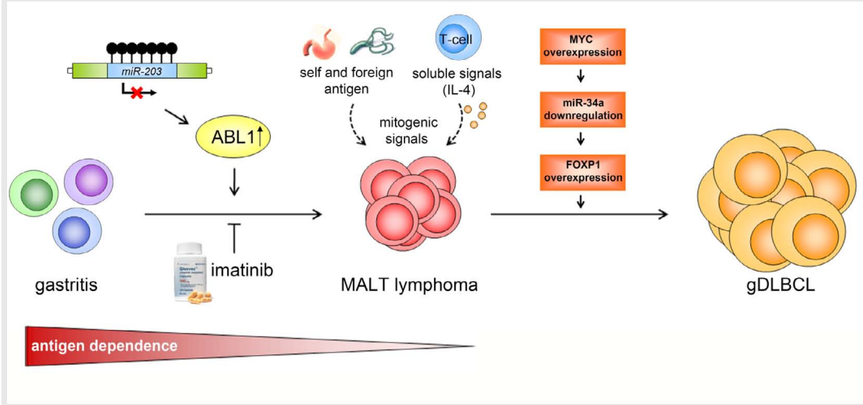

Figure 4.
Tumor cell-intrinsic and extrinsic pathways contribute to gastric lymphoma development upon *Helicobacter pylori* infection.

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

Isabella Toller 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 5). 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.

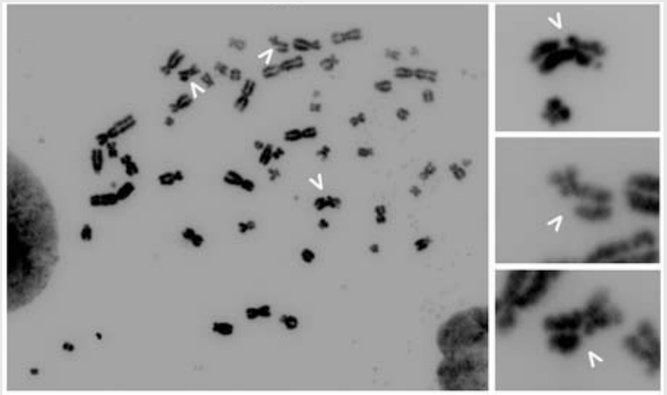

Figure 5.
Helicobacter pylori triggers DNA double strand breaks that are evident in metaphase chromosomes. Panels on the right show broken chromosomes.

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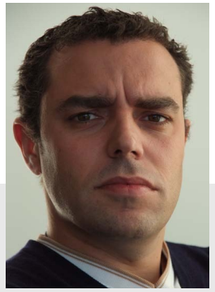

DNA DOUBLE-STRAND BREAK REPAIR AND CANCER

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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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.

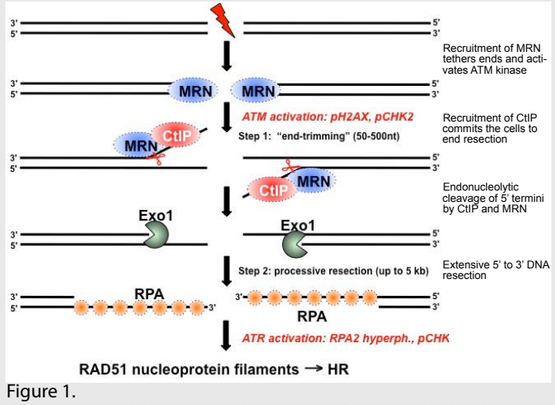

Figure 1.
CtIP is required for DNA end resection. Sensing of DSBs by MRN leads to the activation of ATM and subsequent phosphorylation of several downstream targets involved in DNA damage response (DDR) such as H2AX and CHK2. Initial DNA end resection is realized through MRN and CtIP, followed by extensive 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 Neugebauer

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 Neugebauer

Homologous recombination (HR) and non-homologous end joining (NHEJ) are the two major pathways involved in the repair of DSBs. NHEJ operates throughout the entire cell cycle whereas HR is only active during S/G_2. Repair by NHEJ requires DNA-PK and the XRCC4/DNA ligase IV complex, and is considered as being error-prone given that free DNA ends without sequence homology are directly religated, potentially resulting in genomic translocations. In contrast to NHEJ, HR restores genome integrity in an error-free manner since an intact homologous DNA region is used as a template for repair. HR is initiated by DNA end resection generating stretches of 3' single-strande DNA required for subsequent RAD51-dependent strand exchange. In mammals, DNA end resection is carried out by the MRE11 complex in collaboration with CtIP. The importance of DSB repair in mammalian cells is demonstrated by the tumor predisposition in humans and mice associated with mutation of the HR gene, *Brca1*. Rescue of HR in *Brca1*-deficient cells by deletion of 53BP1 correlates with a significant increase in DSB resection, highlighting the importance of regulation of DNA end resection in determining DSB repair pathway choice. Several recent studies have shown that DNA end resection is regulated by CtIP phosphorylation at different S/T-P sites, consensus motifs for proline-dependent kinases including CDKs. We would like to investigate how the function of CtIP in DSB processing is regulated by phosphorylation. In addition, it is still largely unknown how ubiquitylation controls CtIP function. For instance, it has been reported that CtIP ubiquitylation 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/G_2 phase while its transcript levels remain constant throughout the cell cycle, suggesting that CtIP is regulated by the ubiquitin-proteasome pathway. Ultimately, our goal is to identify the cellular pathways promoting CtIP ubiquitylation and to understand its physiological relevance.

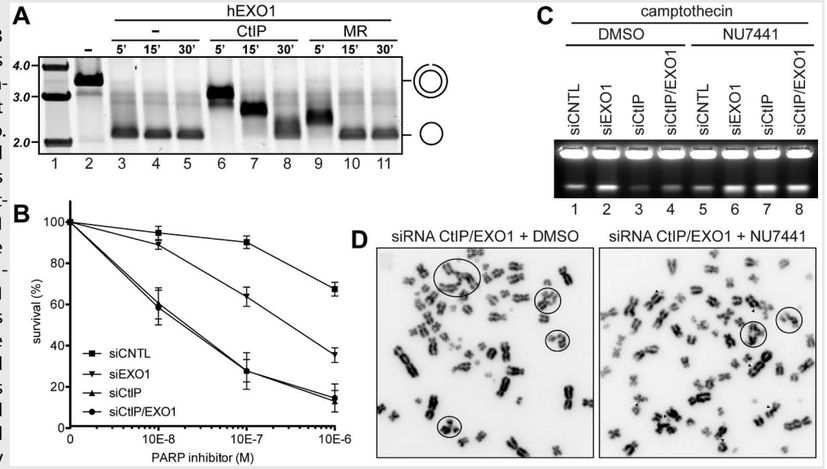

Figure 2.
CtIP and EXO1 cooperate in DSB processing in order to suppress NHEJ-mediated repair of replication-associated DSBs (A) CtIP, but not MR, slows down EXO1 in vitro. Gel-purified pGEM-13Zf(+)-nicked plasmid substrate (3.75 nM) was incubated at 37°C for the indicated time periods with purified EXO1 (15 nM) in the presence or absence of purified CtIP (15 nM) or MRE11-RAD50 (15 nM). (B) CtIP and EXO1 protect against cytotoxic effects of PARP inhibition. 72h after the transfection with the indicated siRNA oligonucleotides, U2OS cells were treated with the indicated doses of AZD2281 (Olaparib) and survival was determined by colony formation. Data represent the mean ± SEM of three independent experiments. (C) U2OS cells transfected with siRNA oligonucleotides were treated with DMSO or camptothecin (2.5 µM, 4h) in presence or absence of 10 µM NU-7441 (DNA-PKcs inhibitor). The amount of broken DNA was assessed by pulsed-field gel electrophoresis (PFGE). (D) Metaphase spreads from cells transfected and treated as in (C). Radial chromosomes (ellipses) and broken chromatids (arrowheads) are indicated. Modified from Eid et al., EMBO Reports 2010.

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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RETO SCHWENDENER

TUMOR-ASSOCIATED MACROPHAGES

Modulation of solid tumors with bisphosphonates and bisphosphonate-liposomes leads to growth inhibition and repolarization to a growth-suppressing tumor microenvironment. We study the potential of this method by two ways: 1) as a tool to study basic biological and immunological effects in the tumor microenvironment and 2) as cancer therapy approach in mouse tumor models. Our aim is to contribute to a better understanding of the complex processes of tumor development, growth and metastatic dissemination.

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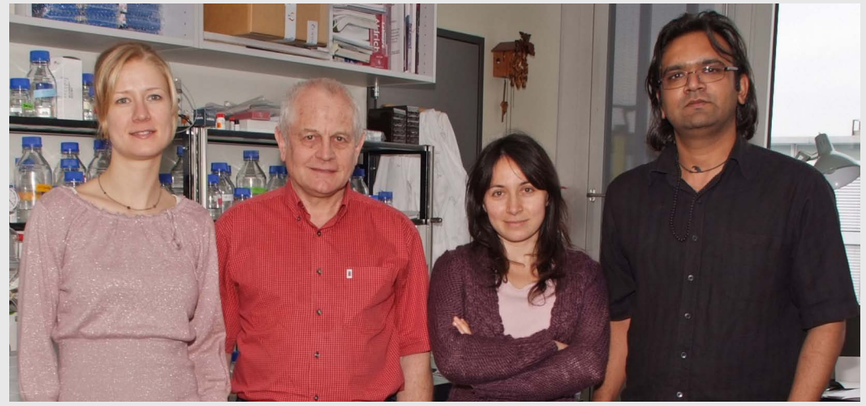

Solid tumors are not only composed of malignant cells; they are complex organ-like structures comprising many cell types, including a wide variety of migratory hematopoietic (macrophages, neutrophils, myeloid-derived suppressor cells, immune cells) and resident stromal cells. Migration of these cell types into tumors has been interpreted as evidence for an immunological response of the host against a growing tumor. It is acknowledged that tumors are largely recognized as "self" and lack strong antigens. Instead, they have the property to manipulate the host immune system to prevent rejection and to facilitate their own growth and spread. This led to the proposal that hematopoietic cell infiltrates have a causal role in carcinogenesis. Clinical data collected from a wide range of solid tumors underscore these results, given that those high densities of leukocytic infiltrations, most notably tumor associated macrophages (TAM) and neutrophils (TAN), correlate with poor prognosis of the diseases.

TAMs are derived from circulating monocytes and are activated macrophages of the polarized type II (M2 macrophages), mainly induced by IL-4, IL-10, IL-13 and corticosteorids. Differential cytokine and chemokine production and coordinated temporal and spatial activities of these cells in the tumor stroma are key features of polarized macrophages that promote tumor angiogenesis and growth. Due to their tumorigenic role, M2-TAMs have been proposed as potential therapeutic targets. Similarly, TANs have been shown assume comparable polarized phenotypes that suppress anti-tumorigenic immune cells in the tumor microenvironment. To study the role of TAMs and TANs we use bisphosphonates and bisphosphonate-liposomes in various subcutaneous mouse tumor models. Tumor growth is monitored by immunohistochemistry, flow cytometry, microscopy and by genomic and proteomic approaches. Additionally, we use in vitro conditioned-medium co-culture models to identify emerging proteins translated in cancer and endothelial cells in response to macrophages.

Most conventional tumor therapies are flawed due to the genetic instability of cancer cells, which leads to drug resistance. Since macrophages and neutrophils are shown to be involved in assisting tumor properties e.g., polarization, invasion and angiogenesis, it is important to reveal the molecular mechanisms responsible for these events. Moreover, it is essential to identify new drug targets in signaling pathways in these cells and apply their inhibitors in liposomal formulations to specifically target myeloid cell types. To achieve this, we have established co-culture models of tumor cells and macrophages, which are used to screen inhibitors of inflammatory signaling pathways. We also exploit our co-culture models to identify nascent proteins translated in cancer and endothelial cells in response to macrophages. This project will shed light on the understanding of the role of the tumor microenvironment and for the development of new therapies.

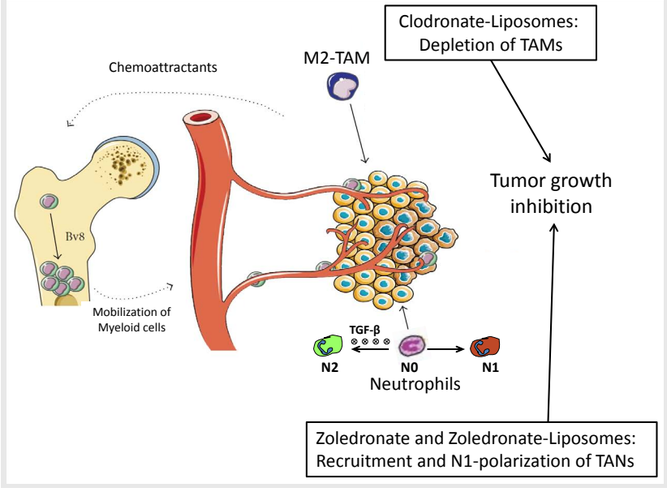

Figure 1.
Recruitment of bone marrow-derived cells such as monocytes/macrophages and neutrophils to the tumor microenvironment. Tumor and stromal cells mobilize tumor promoting myeloid cells to the peripheral blood through secretion of cytokines and chemokines followed by the mobilization and infiltration of these cells to the tumor microenvironment, where they assume tumorigenic properties. Bisphosphonate-liposomes inhibit tumor growth, either by depletion of M2-polarized TAMs or by causing neutrophil infiltration and skewing of N2-neutrophils to anti-tumorigenic N1-neutrophils, thereby creating an anti-tumorigenic microenvironment (Modified from Schmid & Varner, J. Oncol. 2010).

Zoledronate-mediated modulation of the tumor microenvironment leads to impaired tumor growth

Sibel Mete, Sushil Kumar

Zoledronate, an inhibitor of osteoclastic bone resorption, is commonly used to prevent and treat osteoporosis. There is emerging interest in the use of zoledronate as an anticancer agent based on preclinical evidence of its anti-tumor properties. Due to its high affinity for bone matrix, most models addressed the ability of zoledronate to reduce skeletal tumor burden and prevent bone metastases. However, whether zoledronate prevents tumor progression in soft tissue tumors and the mechanism of its antitumor effects is still under investigation.

To address these issues, we treated mice bearing syngeneic subcutaneous tumors with zoledronate and zoledronate-liposomes. A significant reduction in growth of Lewis lung (LLC) and colon carcinoma (MC38), but not B16 melanoma, tumors in mice was observed. We examined the effect of the drug on the tumor microenvironment focusing on tumor infiltrating myeloid cells. We saw an increase of $CD11b^+$ myeloid cells in the tumor microenvironment as well as in spleen, blood and peritoneum of treated animals. It is known that solid tumors actively recruit myeloid cells and divert their functions toward an immune-suppressive and pro-tumorigenic M2-like phenotype. The inverse correlation between myeloid cell density and tumor growth in zoledronate-treated animals points to a reprogramming of these cells: myeloid cells from treated tumors were found to acquire an M1 anti-tumorigenic phenotype, as shown by increased expression of pro-inflammatory and immunostimulatory factors and reduced expression of the immunosuppressive factors. Furthermore, these cells displayed an enhanced ability to stimulate proliferation of naive $CD8^+$ T cells. Further characterization of these cells identified the neutrophils as increasingly accumulating myeloid cell types in tumors of zoledronate-treated animals. Accordingly, zoledronate was found to increase the production of neutrophil-attracting chemokines by cancer cells as well as tumor infiltrating myeloid cells. Further analysis of $CD11b^+Ly6G^+$ neutrophils and $CD11b^+Ly6G^-$ monocytes/macrophages revealed that zoledronate exerts inflammatory and immunogenic transcriptional changes specifically in neutrophils, but not in macrophages. Another key finding was that recombinant TGF-β administration reduced therapeutic efficacy of zoledronate by reducing neutrophil infiltration. To improve the antitumor efficacy of zoledronate, we encapsulated the drug into liposomes, which significantly improved the antitumor efficacy of zoledronate by altering its pharmacokinetics and biodistribution profiles. Collectively, our findings reveal novel anti-tumorigenic properties of zoledronate that may assist in the design of more effective immunotherapeutic approaches for cancer.

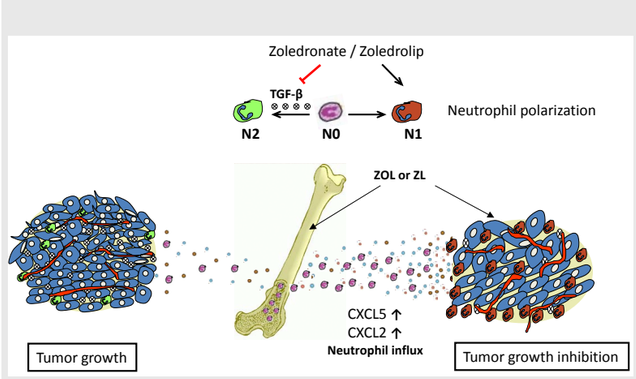

Figure 2.
Model of neutrophil re-polarization induced by zoledronate. Upon in vivo administration, zoledronate preferentially accumulates in bone where it inhibits tumor-induced osteoclast activity and bone resorption. Thereby, zoledronate prevents the release of bone matrix-embedded growth factors (e.g. TGF-β, IGF-1, BMPs) that, induce enhanced expression of neutrophil-attracting chemokines, and thus cause increased accumulation of neutrophils in tumors. Zoledronate skews neutrophil polarization away from the pro-tumorigenic and immunosuppressive N2-like phenotype that is known to be regulated by TGF-β. Accordingly, TGF-β administration reverses the anti-tumor effects. Free zoledronate primarily accumulates in bones while liposomal encapsulation of the drug improves its bioavailability in extraskeletal tumor sites that leads to stronger inhibition of tumor growth.

Tumor-associated macrophages regulate tumor cell malignancy by induction of epithelial to mesenchymal transition

Anne-Katrin Bonde

Macrophages are important components of the tumor microenvironment and their cancer-promoting properties are widely acknowledged. Besides regulating the "angiogenic switch" and remodeling the extracellular matrix, a number of studies have suggested that macrophages orchestrate the migration and invasion of epithelial tumor cells. Epithelial-mesenchymal transition (EMT) is a well-characterized cellular process, through which cells down-regulate epithelial adherence molecules and acquire motile and invasive properties. In this project we are addressing the potential involvement of TAMs in the regulation of an EMT-associated phenotypic shift in tumor cells. We have used liposome-encapsulated clodronate to deplete macrophages in a murine F9-teratocarcinoma model. Gene expression analysis indicated a reduction in mesenchymal gene expression in macrophage depleted tumors. Our data suggest that macrophages can contribute to the regulation of an EMT-associated phenotypic shift in tumor cells. Using conditioned medium culturing we identified macrophage-derived TGF-β as the main regulator of the mesenchymal phenotype in F9-cells and mammary gland NMuMG-cells. Moreover, macrophage conditioned medium, as well as recombinant TGF-β, stimulated the invasive properties of the cells. The clinical relevance of our findings was addressed in a cohort of 491 non-small cell lung cancer patients by an immunohistochemical analysis. This study confirmed a significant correlation between CD68⁺ macrophage density, a pronounced mesenchymal tumor cell profile and tumor grade. In conclusion, this project has identified a regulatory role for TAMs in EMT-associated phenotypic shift of tumor cells.

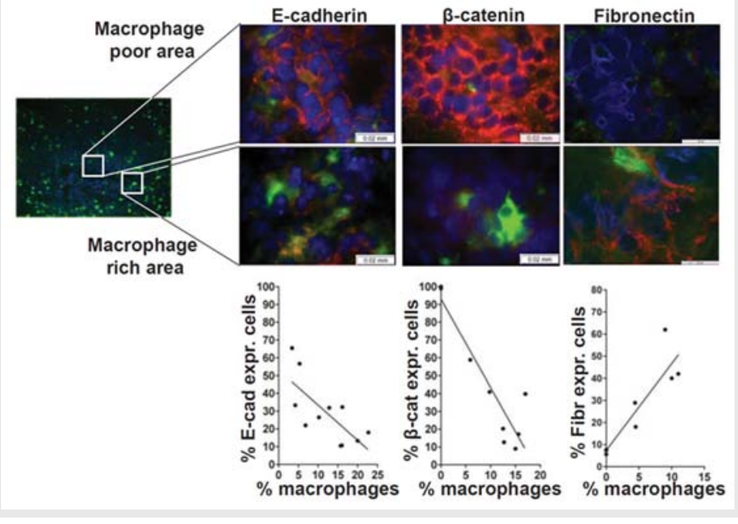

Figure 3.
Immunohistochemical analysis identifies correlations between tumor cell expression of EMT markers and intra-tumoral CD68⁺ macrophage density. Whereas E-cadherin (red, left panel) and β-catenin (red, middle panel) localize to the plasma membrane of tumor cells in areas with low CD68⁺ macrophage (green) densities (upper row), expression of both proteins is compromised and partially lost in areas with high CD68⁺ densities (lower row). Conversely, fibronectin (red, right panel) expression is increased in areas with high CD68⁺ densities. Thus, intra-tumoral TAM density correlates with a mesenchymal tumor cell phenotype in F9-tumors. The nuclei are stained with DAPI (blue). Scale bar=0.02 mm.

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ADMINISTRATIVE, IT AND LABORATORY SUPPORT

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

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Veljkovic, E; Jiricny, J; Menigatti, M; Rehrauer, H; Han, W. Chronic exposure to cigarette smoke condensate in vitro induces epithelial to mesenchymal transition-like changes in human bronchial epithelial cells, BEAS-2B. Toxicology in Vitro, 25(2):446-453.

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Zattoni, M; Mura, M L; Deprez, F; Schwendener, R A; Engelhardt, B; Frei, K; Fritschy, J M. Brain infiltration of leukocytes contributes to the pathophysiology of temporal lobe epilepsy. Journal of Neuroscience, 31(11):4037-4050.

Dissertations

Hitzler, I. Immune mechanisms governing infection control and pathogenesis of helicobacter pylori and prospects for a human vaccine.

Mete, S. Targeting tumor microenvironment by zoledronate as a novel therapeutic approach in cancer.

Tiwari, A. Functional characterization of KIAA1199.

COLLABORATIONS

Academy of Sciences of the Czech Republic, Prague, Czech Republic
Structural studies on the motor subunit of type I restriction enzymes

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

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

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

Deutsches Krebsforschungszentrum, Heidelberg, Germany
DNA damage response

Duke University, Durham, NC, USA
Role of RECQ5 helicase in transcription

École Polytechnique Fédérale de Lausanne, Lausanne, Switzerland
Cancer stem cell biology

Erasmus University of Rotterdam, Rotterdam, The Netherlands
Mismatch repair

ETH Zürich, Zürich, Switzerland
DNA damage response
Colon cancer

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DNA replication fork

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Mismatch repair

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RecQ helicases

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DNA damage response
Mismatch repair

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Liposomes
Helicobacter pilori

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Treg in MALT lymphoma

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

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

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

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

Medizinische Hochschule, Hannover, Germany
DNA damage response

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

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

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

Ospedale di Cremona, Cremona, Italy
Colon Cancer

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 Cambridge, Cambridge, UK
DNA damage response

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

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

University of Kyoto , Kyoto, Japan
Cross-link repair

University of Lausanne, Lausanne, Switzerland
Cancer Stem Cell Biology
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 Roskilde, Roskilde, Denmark
Cellular studies on hEXO1

University of Sussex, Brighton, UK
Mismatch reapir

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

University of Vienna,Vienna, Austria
Mismatch repair

Wellcome Trust Sanger Institute, Cambridge, UK
Helicobacter pilori genome

TEACHING

Ferrari Stefano
DNA metabolism and cancer
Cancer Biology Program UZH: Module A - Cell Signaling and Cell Cycle
Genome Instability and Molecular Cancer Research

Janscak Pavel
DNA metabolism and cancer
Genome Instability and Molecular Cancer Research

Jiricny Josef
DNA metabolism and cancer
DNS Schäden, DNS Reparatur und Krebs
Arbeiten im Institut für Molekulare Krebsforschung
Cancer Biology Program UZH
Cancer Research Club
Diskussion wissenschaftlicher Arbeiten
Diskussion wissenschaftlicher Projekte
Leitung wissenschaftlicher Arbeiten in Molekularer Krebsforschung für Diplomierende und Doktorierende
Repair, Recombination, Replication
Genome Instability and Molecular Cancer Research
Research Internship quantitative and systems Biology
Journal Club Systems Biology / Functional Genomics
Molecular and Cell Biology of Cancer
Mantelstudium Biomedizinische Wissenschaften: Grundlagen I
Kolloquium über Literatur
Lecture Series in Molecular Life Science
Science Ethics

Lopes Massimo
Coordinator and teacher of two courses for Master Students at the UZH:
Genomic Instability and Molecular Cancer Research
DNA metabolism and Cancer

Marra Giancarlo
DNA metabolism and cancer
Genome Instability and Molecular Cancer Research

Müller Anne
Molekulare Zellbiologie I
Molekulare Zellbiologie II
DNA metabolism and cancer
Journal Club Systems Biology, Functional Genomics
Scientific writing for PhD Students
Clinical Cancer Research for PhD students in the Cancer biology program
Genome Instability and Molecular Cancer Research
ETH- Infectious agents: From molecular biology to disease

Sartori Alessandro
Molekulare Zellbiologie I
DNA metabolism and cancer
Genome Instability and Molecular Cancer Research

Schwendener Reto
DNA metabolism and cancer
Genome Instability and Molecular Cancer Research

CONFERENCES

2010

Ferrari Stefano
Cell Cycle and Cancer
Inaugural lecture, University of Zurich

The cellular response to genotoxic damage
Casa Sollievo della Sofferenza, IRCCS, S. Giovanni Rotondo, Italy

The cell cycle: regulatory aspects and the link to cancer
Casa Sollievo della Sofferenza, IRCCS, S. Giovanni Rotondo, Italy

The cell cycle and its regulation
Interdepartmental Center "A. Vallisneri", University of Padua, Italy

The effect of mitotic DNA damage on the TPX2-Aurora A complex
Novartis Institutes for Biomedical Research, Basel, Switzerland

Janscak Pavel
Role of RECQ5 DNA Helicase in Maintenance of Genomic Stability
University of Karlsruhe, Germany

Jiricny Josef
Identification of KIAA1018/FAN1 and its role in interstrand cross-link repair
40 Years FMI, Basel, Switzerland

A new player involved in the processing of cisplatin damage
9th Day of Clinical Research, Zurich, Switzerland

The mismatch repair interactome
Abcam Genomic Instability Meeting, Antigua

Interference of base- and mismatch repair in somatic hypermutation?
AID Workshop-From Immune Diversification to Cancer, Stockholm, Sweden

The mismatch repair interactome or the trouble with highthroughputomics
DKFZ, Heidelberg, Germany

Lopes Massimo
Fork reversal precedes double strand breaks upon Top1 inhibition
EMBO Conference on Recombination Mechanisms, Castelvecchio Pascoli, Italy

Towards the structural visualization of genome instability during DNA replication
Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland

Replicazione del DNA e cancro: verso la visualizzazione dell 'instabilita' genomica associata allo stress replicativo
Institute of Medical Genetics, C.S.S., San Giovanni Rotondo, Italy

Structural insights into genome instability associated with DNA replication stress
International Conference on Radiation and Cancer Biology, Nagasaki, Japan

Towards the structural visualization of genome instability during DNA replication
MRC Protein Phosphorylation Unit, Dundee, UK

Replication fork reversal precedes double strand break formation upon Top1 inhibition
Russian– SwissWorkshop on Regulation of genome stability by DNA replication and repair, St. Petersburg, Russia

Structural insights into genome instability associated with DNA replication stress
ZMBH, University of Heidelberg, Germany

Marra Giancarlo
Reductionist and integrationist routes toward a better understanding of intestinal tumors
2nd workshop on Gastrointestinal Oncology, Sarteano, Italy

Basi Molecolari della Carcinogenesi Colorettale: Quante Strade?
Grandi dibattiti e contorversie nei tumori gastrointestinali, Cremona, Italy

Müller Anne
Tolerance rather than immunity protects from *Helicobacter*-induced gastric preneoplasia
8th International Conference on Pathogenesis and Host response in Helicobacter infections, Lo-skolen, Helsingor, Denmark

Tolerance rather than immunity protects from *Helicobacter*-induced gastric cancer
Fred Hutchinson Cancer Research Center, Seattle, USA

Pathogenesis of gastric *Helicobacter*-associated B-cell lymphoma: cellular players and molecular mechanisms
Novartis Institutes for Biomedical Research, Dept. of Oncology, Basel, Switzerland

Effector and regulatory T-cells and their role in the induction and prevention of *Helicobacter*-associatedgastric malignancies
Sanger Institute of the Wellcome Trust, Cambridge, UK

Sartori Alessandro
CtIP-EXO1 interaction controls DNA end resection in human cells
11th Biennual DGDR Meeting: "Repair Meets Replication", Jena, Germany

Mechanistic insights into the regulation of DNA end resection
Abcam Conference: "Maintenance of Genome Stability", Antigua

DNA double-strand break repair: From Mechanistic Understanding to Cancer Therapy
Inaugural lecture, University of Zurich, Switzerland

CtIP: An evolutionarily conserved protein involved in DNA double-strand break repair
CNRS-CGM, Gif-sur-Yvette, France

Resection of DSBs: It's decision time
FMI, Basel, Switzerland

CtIP controls EXO1-mediated DNA end resection in human cells
Russian-Swiss Workshop: "Regulation of Genome Stability by DNA Replication and Repair", St. Petersburg, Russia

Molecular Oncology: Understanding the links between DNA damage, repair and cancer
Vontobel-Stiftung, Orelli-Saal, University of Zurich, Switzerland

2011

Ferrari Stefano
Exonuclease-1 and genome stability
Center for Integrative Biology, University of Trento, Italy

Janscak Pavel
Cooperation between Werner Syndrome Protein and DNA Polymerase lambda in Repair of Oxidative DNA Damage
International Centre for Genetic Engineering and Biotechnology, Trieste, Italy

Role of Werner Syndrome Protein in Repair of Oxidative DNA Damage
Istituto Superiore di Sanità, Rome, Italy

Jiricny Josef
Identification of KIAA1018/FAN1 and its role in interstrand cross-link repair
10th Charles Rodolphe Brupbacher Symposium, Zurich, Switzerland

The mismatch repair interactome
4th EU-USA Conference on Base Damage and Repair, Oslo, Norway

Interplay of BER and MMR in somatic hypermutation
The Enemy Within: Endogenous DNA Damage as a Source of Cancer and Ageing, Baeza, Spain

The mismatch repair interactome
Clare Hall, Cancer Research UK, South Mimms, UK

The mismatch repair interactome
Genomic Stability Center, Brighton, UK

The mismatch repair interactome
Gordon Conference on Genetic Toxicology, Il Ciocco, Italy

The mismatch repair interactome
IDI, Rome, Italy

FAN1 - a novel enzyme involved in the processing of cisplatin adducts in DNA
Institute of Hematology, USZ, Zurich, Switzerland

FAN1 - a novel enzyme involved in the processing of cisplatin adducts in DNA
Institute of Clinical Chemistry, USZ, Zurich, Switzerland

FAN1 - a novel enzyme involved in the processing of cisplatin adducts in DNA
National Cancer Institute Congress, Liverpool, UK

Mismatch repair proteins in cross-link repair and antibody maturation
Responses to DNA damage: from molecular mechanism to human disease, Egmond aan See, Netherlands

The mismatch repair interactome
University of Milano, Italy

Lopes Massimo
Structural and molecular insights into DNA replication stress
Biotech Research and Innovation Center (BRIC), University of Copenhagen, Denmark

Structural and molecular insights into DNA replication stress
Centro Nacional de Investigaciones Oncologicas , Madrid, Spain

Replication fork reversal limits double strand break formation upon Top1 inhibition
Clare Hall Institute, Cancer Research UK, UK

Structural and molecular insights into DNA replication stress
Columbia University, New York, USA

Structural insights into DNA replication stress
FASEB conference on "Genetic Recombination and Genome Rearrangements", Steamboat Springs, Colorado, USA

Towards the structural visualization of genome instability during DNA replication
International Center of Genetic Engineering and Biotechnology, Trieste, Italy

Structural and molecular insights into DNA replication stress
Meeting "Image the DNA damage response", University of Milano, Bicocca, Italy

Structural insights into oncogene-induced DNA replication stress
Workshop "The Enemy Within: Endogenous DNA Damage as a Source of Cancer and Ageing", Universidad Internacional de Andalucia, Baeza , Spain

Marra Giancarlo
Reductionist and integrationist routes toward a better understanding of colorectal tumorigenesis
1st Staromics Retreat, Leysin, Switzerland

Reductionist and integrationist routes toward a better understanding of colorectal tumorigenesis
University of Innsbruck, Austria

Müller Anne
Tolerance rather than immunity protects against gastric cancer precursor lesions
19th United European Gastroenterology Week, Stockholm, Sweden

Neonatally induced immunological tolerance to *Helicobacter pylori* protects mice against gastric preneoplasia and experimentally induced asthma
7th Conference on New Frontiers in Microbiology and Infection, Villars-sur-Ollon, Switzerland

Tolerogenic dendritic cells drive Treg differentiation and induce *Helicobacter pylori*-specific immune tolerance and protection against asthma
Conference on Campylobacter, Helicobacter and related organisms (CHRO), Vancouver, Canada

Helicobacter pylori targets dendritic cells to induce immune tolerance and protection against allergic asthma
Department of Dermatology, University Hospital Zurich, Switzerland

Helicobacter pylori-associated gastric malignancies: cellular players and molecular mechanisms
Institute of Pathology, University of Basel, Switzerland

Tolerogenic dendritic cells drive Treg differentiation and induce *Helicobacter pylori*-specific immune tolerance and protection against asthma
Institute of Pharmaceutical Sciences, ETH Zürich, Switzerland

Helicobacter pylori-specific immunological tolerance protects against gastric cancer precursor lesions and asthma
Max Planck Institute for Infection Biology, Berlin, Germany

Neonatally induced immunological tolerance to *Helicobacter pylori* protects mice against gastric preneoplasia and experimentally induced asthma
Technical University of Munich, Institute of Microbiology, Munich, Germany

Neonatally induced immunological tolerance to *Helicobacter pylori* protects mice against gastric preneoplasia and experimentally induced asthma
The Sahlgrenska Academy at Göteborg University, Sweden

Helicobacter pylori-specific immunological tolerance protects against gastric cancer precursor lesions and asthma
World Immune Regulation Meeting, Davos, Switzerland

Tolerance rather than immunity protects from *Helicobacter*-induced gastric preneoplasia
XXVth International Workshop on *Helicobacter* and related bacteria in chronic digestive inflammation and gastric cancer, Dublin, Ireland

Sartori Alessandro
The prolyl-isomerase PIN1 interacts with CtIP: Implications for DSB repair pathway choice
27th RBC-NIRS international symposium: "Chromatin dynamics and epigenetic memory in DNA damage response", Kyoto, Japan

Multi-site phosphorylation control CtIP function in DSB repair
Invited Seminar, DKFZ, Heidelberg , Germany

Schwendener Reto
Liposomes in Biology and Medicine: From Biomembranes to Drug Delivery Vehicles
Institute of Physical Chemistry, University of Zurich, Switzerland

Bisphosphonate liposomes and their role in tumor therapy
Translational Oncology, Johannes Gutenberg-University, Mainz, Germany

Mouse physiology and pathophysiology: Mouse tumor models
Zurich Center for Integrative Human Physiology (ZIHP), University of Zurich, Switzerland

SEMINARS

2010

Blasius, Melanie
Wellcome Trust/Cancer Research UK Gurdon Institute, Cambridge, UK
A screen for novel substrates of the checkpoint kinase

Boulton, Simon
DNA damage Response Laboratory, Cancer Research UK, Clare Hall Laboratories, UK
Regulating recombination during replication and meiosis

Crouse, Gray F.
Department of Biology, Emory University Atlanta, USA
DNA mismatch repair in yeast

Filippov, Dmitri V.
Leiden Institute of Chemistry, Leiden University, The Netherlands
Synthesis of Mono-ADP-Ribosylated Oligopeptides Using Ribosylated Amino Acid Building Blocks

Gerlich, Daniel
Institute of Biochemistry, University of Zurich, Switzerland
A cytokinetic abscission checkpoint protects human cells against tetraploidization

Giannattasio, Michele
Department of Biomolecular Sciences and Biotechnology, University of Milano, Italy
UV-induced checkpoint response in non-replicating yeast cells: from the genetic dependences to the structural determinants

Hiom, Kevin
Biomedical Research Centre, University of Dundee, UK
The role of BRCA1 in repairing DNA breaks

Junya, Kobayashi
Department of Genome Repair Dynamics, Radiation Biology Center, Kyoto University, Kyoto, Japan
The role of novel H2AX-binding factor, nucleolin in DNA damage response

Paro, Renato
Department of Biosystems Science and Engineering (D-BSSE) ETH Zurich, Basel, Switzerland
Signaling and epigenetic reprogramming during tissue regeneration

Pasero, Philippe
IGH Institute of Human Genetics, Montpellier, France
Cellular responses to replication stress in yeast and human cells

Petrini, John H. J.
Laboratory of Chromosome Biology, Memorial Sloan-Kettering Cancer Center New York, USA
Genetic analysis of the DNA damage response

Schmid, Roland
Director, II. Medical Clinic and Polyclinic in Munich, Germany
Identification of targets for prevention and treatment of pancreatic cancer

Sturla, Shana J.
ETH Zürich, Department of Agricultural and Food Sciences, Switzerland
Probing DNA damage in carcinogenesis and cancer therapy with small molecules

van der Horst, G.T.J.
Dept. of Cell Biology and Genetics, Erasmus Rotterdam, The Netherlands
Circadian clock, DNA damage and cancer

Vindigni, Alessandro
International Centre for Genetic Engineering and Biotechnology, Trieste, Italy
Understanding the multiple roles of RecQ helicases in genome maintenance

2011

Bass Adam
Dana-Farber Cancer Institute, Boston, Massachusetts, USA
Emerging Lesson From Genomic Characterization of Gastrointestinal Cancer

Blow J. Julian
Wellcome Trust Centre for Gene Regulation & Expression University of Dundee, UK
How S phase is organised to ensure complete genome duplication, and why cancer cells might get it wrong

Cejka Petr
Department of Microbiology, UC Davis, USA
Biochemical characterization of the Sgs1 helicase: from DNA end resection to processing of recombination intermediates

Cotta-Ramusino Cecilia
Harvard University Medical School, Boston, USA
Genetic Interrogation of the DNA Damage Response

Doherty Aidan
MRC Genome Damage and Stability Centre University of Sussex Falmer, Brighton, UK
Orchestration of DNA Double-Strand Break Repair Processes by NHEJ Polymerases

Egly Jean-Marc
Institute of Genetics and Molecular and Cellular Biology, France
The NER factors in transcription

Hardt Wolf Dietrich
Institut of Microbiology, ETHZ, Switzerland
Salmonella Typhimurium: analyzing host cell manipulation in vivo

Hirota Kouji
Kyoto University, Japan
DNA Polymerase delta efficiently inserts nucleotides opposite abasic sites in vivo

Longhese Maria Pia
Dept. of di Biotechnology and Biosciences, University of Milano Bicocca, Milano, Italy
Processing and repair of DNA double-strand breaks to maintain genome integrity

Lopez-Bigas Nuria
Research Unit on Biomedical Informatics, University Pompeu Fabra, Barcelona, Spain
Integration and data-mining of multidimensional oncogenomics data

Lukas Jiri
Center for Genotoxic Stress Research, Danish Cancer Society, Denmark
Chromatin response to DNA damage and replication stress: Mechanisms, and a new role in shielding fragile genomic loci

Meraldi Patrick
Department of Biology, ETH Zürich, Switzerland
Ensuring genomic stability during human cell division

Muzi-Falconi Marco
Dipartimento di Scienze Biomolecolari e Biotecnologie Universita' degli Studi di Milano, Italy
A new role for Exo1 in a conserved mechanism for responding to UV irradiation

Penengo Lorenza
Laboratory of Molecular Biology, DiSCAFF Dept., Novara, Italy
To UB or not to UB? When UBiquitin makes the choice: the case of RNF168

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

Schärer Orlando D.
Pharmacological Sciences and Chemistry, Stony Brook University, NY, USA
Regulating endonuclease activity in human DNA repair pathways

Sommer Suzanne
Institute de Génétique et Microbiologie, Université Paris-Sud, Orsay, France
New insights into the mechanisms involved in the extreme radioresistance of the bacterium Deinococcus radiodurans

Storici Francesca
Georgia Institute of Technology Atlanta, USA
Mechanisms of information flow from RNA to DNA

Torres-Padilla Maria Elena
Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC) Illkirch, France
Epigenetic mechanisms in early mammalian development

AWARDS

Josef Jiricny was awarded the Ernst Th. Jucker Prize 2010.

IMCR PERSONNEL

Postdoctoral Research Assistants

Isabelle Arnold*
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Lorenzo Lafranchi Lepakshi
Maryna Levikova
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Eveline Bergmüller
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Bettina Rausch-Malina
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Visiting Scientists

Kouji Hirota
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Visiting Students

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Jana Langhoff
Manuel Noben
Judith Oehler
Yukako Sakagami
Mirjam Schneider
Temel Sevcan
Andreas Sturzenegger
Kyoko Sugimura
Vaclav Urban
Clément Vuarchey
Isabella Zanini
Zuzana Zigova
Maurice Zingg

* left 2010/11