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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).

In August of 2008, we were rejoined by Alessandro A. Sartori, an alumnus, who has returned to us from his extended postdoctoral stay in the Wellcome/CRC Gurdon Institute in Cambridge. One year later, Alex was appointed as an Assistant Professor, a new position generously supported by the Vontobel Trust. With additional support from the Swiss National Science Foundation, Alex has already built-up a small group that will study the molecular mechanisms of double strand break repair in human cells.

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 proven to be extremely popular, and which has grown into the third largest PhD Program in Zurich. It recruits top students from all over the world not only into the IMCR, but also into the research groups of the Cancer Network Zurich (www.cnz.unizh.ch) and other research groups of the University and the Swiss Federal Institutes of Technology (ETH).

With the arrival of the Sartori group and the new student intake, the Institute has now reached its full capacity. It houses nearly 70 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.

Our long-term goal is to position ourselves among the leading institutes of molecular cancer research in the world, an effort that would be greatly facilitated by the creation of a second Chair of Cancer Research. In the current economic climate, this dream can only be realised with the help of external sponsors.

In this report, we provide the reader with an overview of our current research efforts.

We look forward to another period of exciting and innovative 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

Scientific Advisory Board

Susan Gasser (Friedrich Miescher Institute, Basel, Switzerland)

Walter Gehring (University of Basel, Switzerland)

Tomas Lindahl (Clare Hall Laboratory of Cancer Research, UK).

Stefano Ferrari

DNA damage and the regulation of cell division

DNA damage seriously threatens the genome. Failure to correct damage before cell division promotes genetic instability, a common trait of cancer cells. To maintain genome stability, a network of proteins has evolved with the function of sensing and repairing DNA damage. Lesion processing and DNA repair are accompanied by the generation of signals that delay the onset of mitosis. This is known as the "DNA damage response" (DDR).

We are interested in elucidating the wiring of checkpoint pathways that are activated in response to DNA damage. Specifically, two aspects of the DDR are under scrutiny: (i) the clarification of mechanisms that control components of the machinery dedicated to resection of double-strand breaks (DSBs); (ii) the effect of DNA damage on the entry and transition through mitosis.

This approach should provide a proof-of-principle to the suitability of targeting checkpoints in cancer therapy.

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Wassim Eid
Kim Engels

Undergraduate Student
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Technician
Christiane König

Regulation of human EXO1 in response to replication fork stalling

Mahmoud El-Shemerly

Human Exonuclease 1 (EXO1) belongs to the RAD2 family of nucleases and was shown to participate in mismatch repair, DNA replication and homologous recombination. Previous work from our laboratory showed that, in response to stalled replication, EXO1 is phosphorylated and, as a consequence, undergoes ubiquitylation and degradation. Using chemical inhibitors, RNA interference or ATM- and ATR-deficient cell lines, we were able to show that, under these conditions, EXO1 phosphorylation is ATR-dependent. We found that EXO1 is phosphorylated at 9 sites in non-stressed cells and that inhibition of DNA synthesis led to phosphorylation of 3 additional sites.
Collectively, our data and evidence published in the literature indicate that uncoupling of DNA synthesis and DNA unwinding at stalled forks generates structures that are suitable for processing by EXO1. Such intermediates, in turn, trigger ATR/CHK1-dependent signals leading to EXO1 phosphorylation and degradation as a means to stabilize stalled forks (Fig. 1).
Ongoing work in the laboratory aims at uncovering all kinases responsible for EXO1 phosphorylation as well as the ubiquitylation pathway controlling EXO1 degradation.

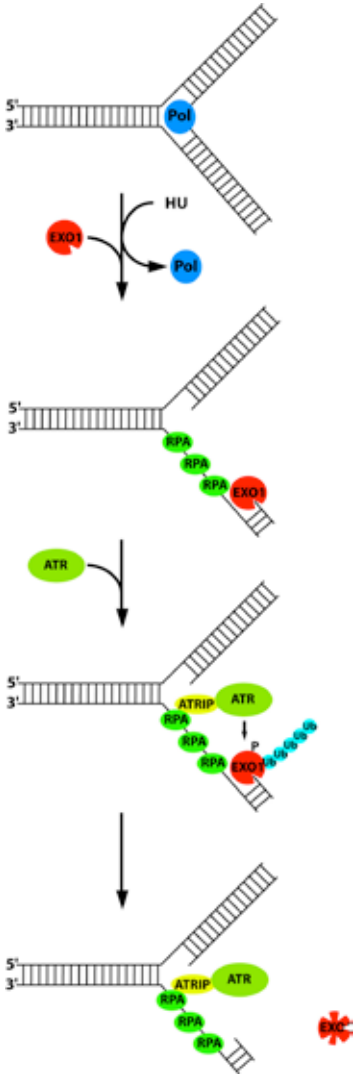

Figure 1.
Schematic model of the control of EXO1 at stalled replication forks.

Identification of EXO1 interacting proteins: a genetic screen

Kim Engels (in collaboration with Massimo Lopes)

To identify proteins interacting with human EXO1, we took advantage of the yeast 2-hybrid assay. A construct encompassing EXO1 C-terminus (ΔN-EXO1) fused to the DNA binding domain of the LexA transcription factor (DBD) was used to screen a human blood peripheral cDNA library. We identified chromatin assembly factors and replication fork-associated proteins, among a number of EXO1 interactors. A replication fork-binding protein was selected for further analysis using budding yeast as model system. Upon confirming the interaction of the yeast homologues of human EXO1 (Exo1) and the fork-associated protein, we used neutral-neutral two-dimensional electrophoresis (2D gel) and electron microscopy (EM) to investigate the biological significance of this interaction. We found that the fork-associated protein was able to restrict access of Exo1 to newly synthesized replication intermediates, thus impairing its ability to generate large ssDNA gaps. Such control appeared to be mediated by the DNA damage-dependent phosphorylation of Exo1 and an altered regulation of Rad53 phosphorylation.

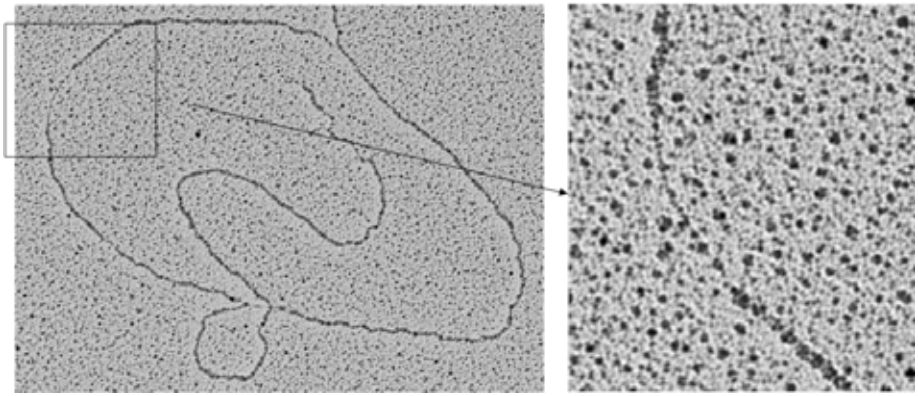

Figure 2. Representative EM image visualizing replication fork intermediates at magnification 66'000x. The inset shows a ssDNA gap located behind the fork of replication.

Regulation of EXO1 activity during resection of double-strand breaks

Wassim Eid, Mahmoud El-Shemerly, Christiane König (in collaboration with Alessandro Sartori, Martin Steger and Emanuele Valtorta)

Investigation of the molecular mechanisms of DNA end resection has recently gained much attention. Studies conducted in different organisms led to the proposal of a two-step model of DSB processing, according to which initial resection ("end-trimming") carried out by MRN and CtIP results in intermediates that serve as templates for long-range DNA end resection. This second, processive step of resection occurs by two alternative mechanisms depending either on EXO1 or BLM.

We found that EXO1 interacts with CtIP and that localization of EXO1 to DSBs depends on MRE11 and CtIP. Functionally, the interaction of CtIP with EXO1 leads to modulation of the exonucleolytic activity of the latter. We are currently addressing the effect of EXO1 depletion in CtIP-downregulated cells using as read-out the sensitivity to DNA damaging agents and the occurrence of gross chromosomal aberrations.

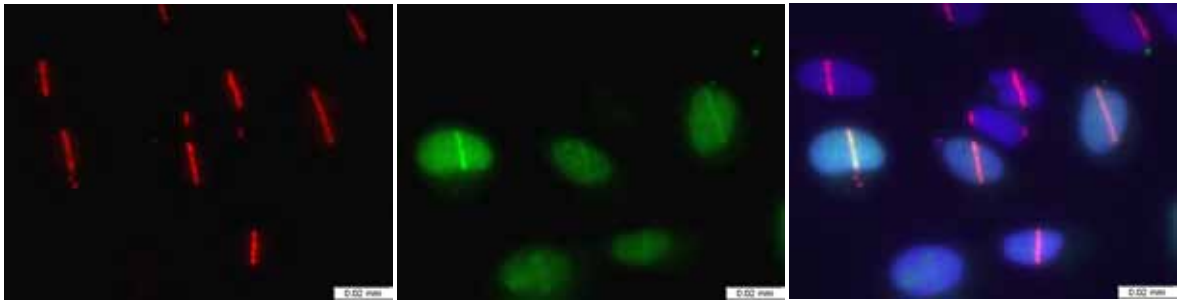

Figure 3. U2OS cells that stably express GFP-EXO1 were microirradiated using an UV-A laser, fixed and stained with an antibody to γH2AX (red). Nuclei were visualized with DAPI.

DNA damage response and transition through mitosis

Payal Bhatia

Transition through mitosis is an obligatory step in the cell cycle of normal and transformed cells. Mitotic kinases are the ultimate target of pathways sensing genotoxic damage and impinging on the cell cycle machinery. The mitotic protein kinase Aurora-A (AurA) is a proto-oncogene normally responsible for separation of duplicated centrosomes and organization of the mitotic spindle at the onset of mitosis.

We have previously shown that AurA is target of DDR pathways at the G2/M transition. In order to dissect the mechanism of AurA inhibition by genotoxic agents, we examined cells irradiated during their transition through mitosis, a time when AurA protein and kinase activity reach peak levels. Ionizing radiation caused a slower metaphase-to-anaphase transition, which we could confirm to depend on ATM activation and not to impair mitotic exit. In irradiated cells, the inhibition of AurA kinase activity was caused by loss of interaction with the activating partner TPX2, with consequent dephosphorylation of AurA T-loop residue T288. The rapid destruction of TPX2 that occurs at mitotic exit appeared to be increased upon irradiation. Detailed analysis, however, revealed that ionizing radiation did not affect the net rate of TPX2 degradation but selectively blocked TPX2 mRNA translation, thus leading to an overall decrease of the TPX2 protein pool. Current experiments are aimed at dissecting the mechanism controlling TPX2 mRNA translation in irradiated cells.

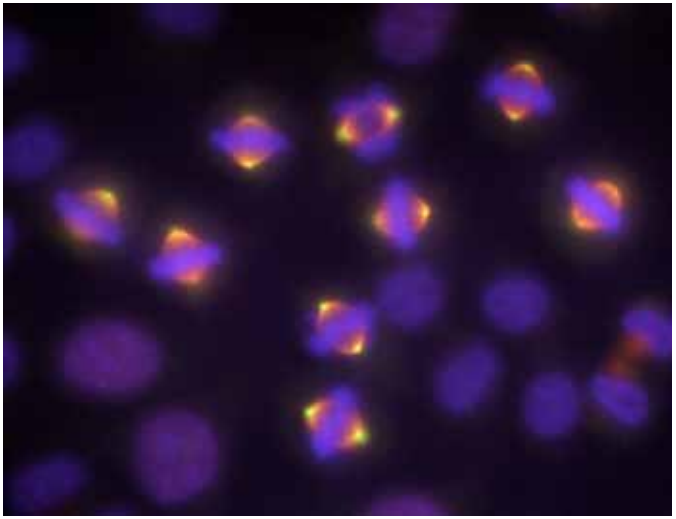

Figure 4. Immunofluorescence image of Hela cells at metaphase (magnification 60x). Spindles were stained in red (TPX2), centrosomes in green (AurA) and DNA in blue (DAPI). The merged signal (yellow) shows co-localization of AurA and TPX2.

These projects were supported by the Swiss National Science Foundation, the Désirée und Niels Yde Stiftung, Krebsliga Zürich, the Ida-de Pottère-Stiftung , the University of Zurich Priority Program (URPP), the Herzog-Egli Stiftung, the UBS Foundation, the Hartmann-Müller Stiftung, the Novartis Foundation for Medical and Biological Research.

Pavel Janscak

Genome maintenance mechanisms

Genomic instability is a hallmark of tumorigenesis and tumor progression. It can be induced by DNA lesions generated either as by-products of endogenous nuclear processes or as a direct consequence of DNA-damaging agents. Research in our laboratory focuses on understanding the molecular mechanisms underlying the biological processes that ensure genomic stability.

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Regulation of homologous recombination

Sybille Schwendener, Shreya Paliwal

Homologous recombination (HR) provides the most accurate mechanism for repair of DNA double-strand breaks (DSBs) and single-stranded (ss) gaps during S/G2 phases of the cell cycle when undamaged sister chromatid can serve as a template for repair. However, HR can be also harmful as inappropriate or untimely HR events can give rise to lethal recombination intermediates and chromosome rearrangements. A critical step of HR is the formation of a RAD51 filament on ssDNA, which mediates the invasion of a homologous DNA molecule. In mammalian cells, several DNA helicases, namely RECQ5, RTEL1 and FBH1, have been implicated in the regulation of initial steps of HR. Our earlier studies have revealed that RECQ5, a member of the RecQ family of DNA helicases, interacts physically with the RAD51 recombinase and disrupts RAD51 presynaptic filaments in a reaction dependent on ATP hydrolysis. More recently, we have precisely mapped the RAD51-interacting domain of RECQ5 and generated mutants that fail to interact with RAD51. We have shown that these mutants are impaired in their ability to displace RAD51 from ssDNA. Moreover, we have found that ablation of RECQ5-RAD51 complex formation by a point mutation alleviates the inhibitory effect of RECQ5 on HR-mediated DSB repair in human cells. These findings provide support for the proposal that interaction with RAD51 is critical for the anti-recombinase attribute of RECQ5. Our current research focuses on the mechanisms that control the anti-recombinase activity of RECQ5 in human cells.

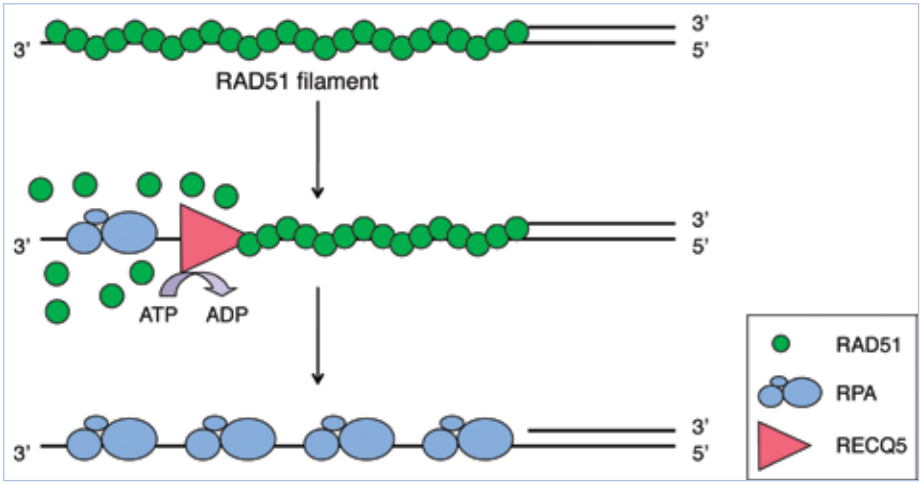

Figure 1. A model for the role of RECQ5 DNA helicase in the regulation of homologous recombination.
RECQ5 utilizes the free energy from ATP hydrolysis to catalyze the disruption of the RAD51 presynaptic filament. The ssDNA generated as a result of RAD51 removal is immediately occupied by RPA to prevent the reloading of RAD51.

Defending genome integrity during transcription

Kanagaraj Radhakrishnan, Daniela Hühn, Stefano Di Marco

Transcription can induce DNA recombination from bacteria to humans, thus affecting genome stability. This phenomenon, referred to as transcription-associated recombination (TAR), has been linked to replication fork pausing resulting from interference between transcription and replication. TAR has also been linked to the formation RNA:DNA hybrids between the nascent transcript and the template DNA strand, which increases the susceptibility of the non-transcribed strand to damage or to the formation of secondary structures that impair replication fork progression. The mechanisms that prevent TAR are not well understood. We and others have shown that RNA polymerase II (RNAP II) stably associates with the RECQ5 anti-recombinase in human cells. More recently, we have found that RECQ5 binds to Ser-2/Ser5-phosphorylated C-terminal repeat domain (CTD) of the largest subunit of RNAP II, RPB1, by means of a Set2-Rpb1-interacting (SRI) motif located at the C-terminus of RECQ5. Furthermore, we have demonstrated that RECQ5 associates with RNAP II-transcribed genes in a manner dependent on its SRI motif. In addition, we have found that RECQ5 density on transcribed genes correlates with the density of Ser2/Ser5-CTD phosphorylation that is associated with the productive elongation phase of transcription. Our future studies will address the possible role of RECQ5 in suppression of TAR.

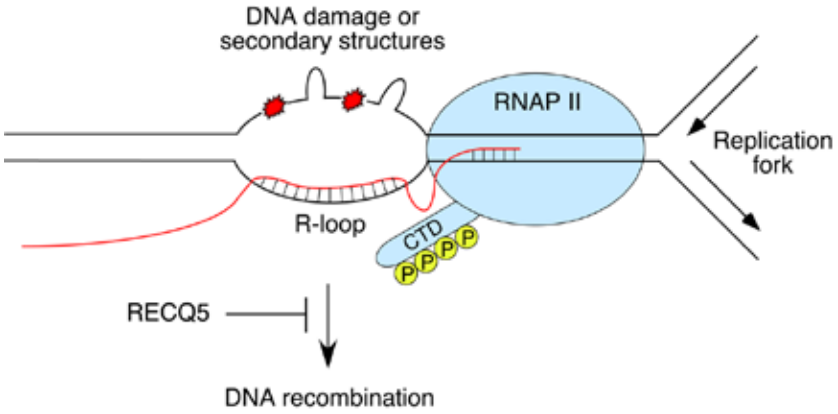

Figure 2. Potential causes of transcription-associated recombination. RECQ5 is proposed to prevent genomic instability during transcription through its association with hyperphosphorylated C-terminal repeat domain (CTD) of RNA polymerase II (RNAP II).

Role of the mismatch repair system in the cellular response to DNA double-strand breaks

Boris Mihaljevic

Proteins involved in the initiation of postreplicative DNA mismatch repair (MMR) such as MSH2, MSH6, MLH1 and PMS2, act during DSB repair to suppress recombination between divergent sequences. Genetic studies in yeast indicated that heteroduplex rejection during DSB repair by the single-strand annealing (SSA) pathway of HR requires the mismatch-binding and ATPase functions of the Msh2p/Msh6p heterodimer and the helicase activity of Sgs1, suggesting a model in which the MMR proteins act in conjunction with Sgs1 to unwind DNA recombination intermediates containing mismatches. In agreement with this model, we found that the human MSH2/MSH6 heterodimer, termed MutSα, physically interacts with the WRN helicase and stimulates WRN-mediated unwinding of forked DNA structures with a 3'-single-stranded arm that form during SSA. More recently, we have found that, like WRN, MMR proteins rapidly accumulate at tracks of DSBs generated by UVA laser in pre-sensitized human cells. Our current studies aim to define the factors that govern the recruitment of MMR proteins to sites of DSBs in human cells and to explore the exact role for the MMR system in DSB signaling and repair.

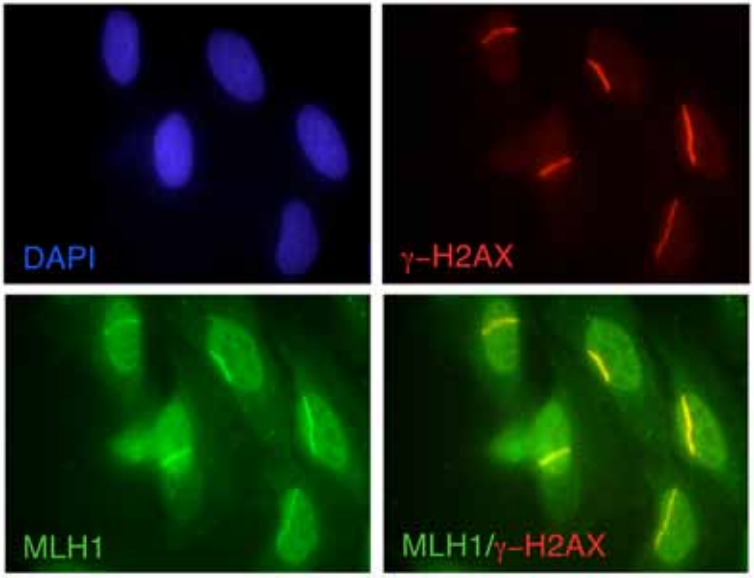

Figure 3. Accumulation of MLH1 at tracks of DNA double-strand breaks induced by UVA laser in pre-sensitized human cells.

These projects are supported by Swiss National Science Foundation, the Swiss Cancer League, the Cancer League of the Canton Zurich, Bonizzi-Theler Stiftung, UBS AG and Lydia Hochstrasser Stiftung.

Josef Jiricny

The multifaceted mismatch repair

During the past 15 years, our 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, recent evidence, both genetic and biochemical, implicated MMR proteins also in other pathways of DNA metabolism and we are now changing direction and focussing on some of these processes.

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Silvia Schanz
Barbara Schöpf

Technician
Mariela Artola-Boran

Biochemistry of mismatch repair

Medini Ghodgaonkar, Patrick Kehl, Kalpana Surendranath

The MMR system requires two sets of proteins: those dedicated to MMR and those that participate also in DNA replication. We have successfully expressed the MMR-specific factors either in the baculovirus system or in E. coli. The replication-specific proteins are also being expressed, even though the multi-subunit nature of some of these factors (e.g. RFC, polymerase-δ) makes the purification strategies difficult. The minimal MMR system could be reconstituted in the laboratories of Paul Modrich and Guo-Min Li some time ago, but there are a number of mechanistic issues that remain to be resolved and this makes it imperative that the recombinant system is working also in our laboratory. One of the aspects we are addressing regards the roles of the various enzymatic activities (ATPases, exonucleases and endonucleases) in the MMR process and in other pathways of DNA metabolism in which these polypeptides are implicated. To this end, we have assembled a number of site-directed mutants lacking these activities. We are now using these variants in the in vitro repair assays and testing how these mutations affect the repair process by studying changes in repair efficiency and in assembly of the repairosome.

Analysis of protein/protein interactions taking place during the repair process has shown that several of the components of the MMR pathway are post-translationally modified. It is currently unclear whether these modifications are constitutive or whether they are regulated, for example during the cell cycle as in the case of EXO1 (see report of Stefano Ferrari) or MSH6 (our unpublished data). As the minimal reconstituted MMR system does not display quite the same substrate specificity as that observed in cell-free systems or in vivo, we postulate either that these differences are due to post-translational modifications of the participating proteins, or that some non-essential components of the MMR pathways are still unidentified.

Using tandem affinity purifications (TAP-tagging) with extracts of stably-transfected cell lines expressing TAP-hMLH1 or TAP-hPMS2, we were able to identify all the known partners of hMLH1 and hPMS2, as well as a number of other polypeptides that apparently do not participate in MMR. Some of these, e.g. FANCJ and REND1, are under study in the laboratory (see below).

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 recombinant lacking the 3′→5′ proofreading activity. The mutator phenotype of these cells is currently under investigation.

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 mutagenic 6-*O*-methylguanine (^{Me}G), such that MMR-deficient cells are up to 100-fold more resistant to killing by methylating agents of the SN1 type than their MMR-proficient counterparts. 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 open-circular 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.

MMR proteins in other pathways of DNA metabolism

Stephanie Felscher, Javier Peña-Diaz, Silvia Schanz

Genetic evidence implicates MMR proteins in other metabolic processes. Thus, sterility of *Mlh1*$^{-/-}$ and *Pms2*$^{-/-}$ mice suggested a role for these polypeptides in meiotic recombination. Similarly, knock out mouse models implicated *Msh2, Msh6* and *Exo1*, but not *Mlh1* or *Pms2* in two key processes of antibody maturation - somatic hypermutation (SHM) and class switch recombination (CSR). The two latter processes are triggered by induction in stimulated B-cells of activation-induced deaminase (AID), which converts cytosines in certain sequence contexts to uracils. The processing of the latter bases by uracil DNA glycosylase (UDG) and the base excision repair process appears to give rise to mutations, unlike in other cell types, where the repair process is error-free. Using defined uracil-containing substrates, we could show that base excision repair and MMR interfere with one another during the processing of substrates

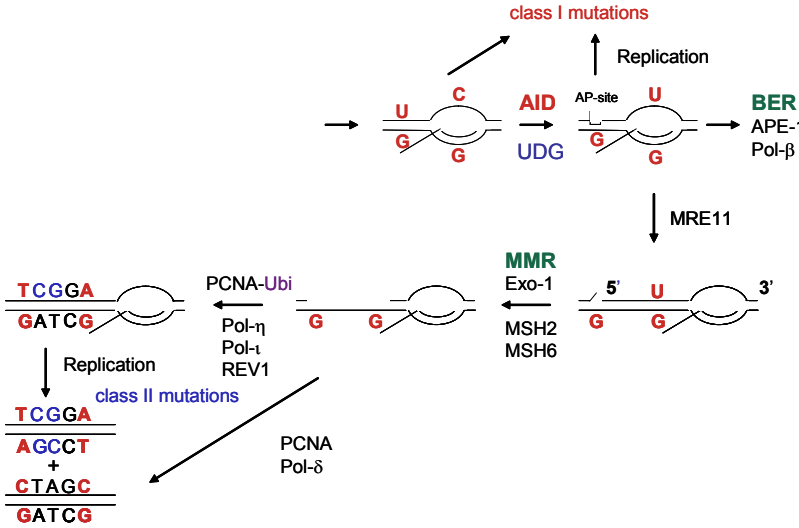
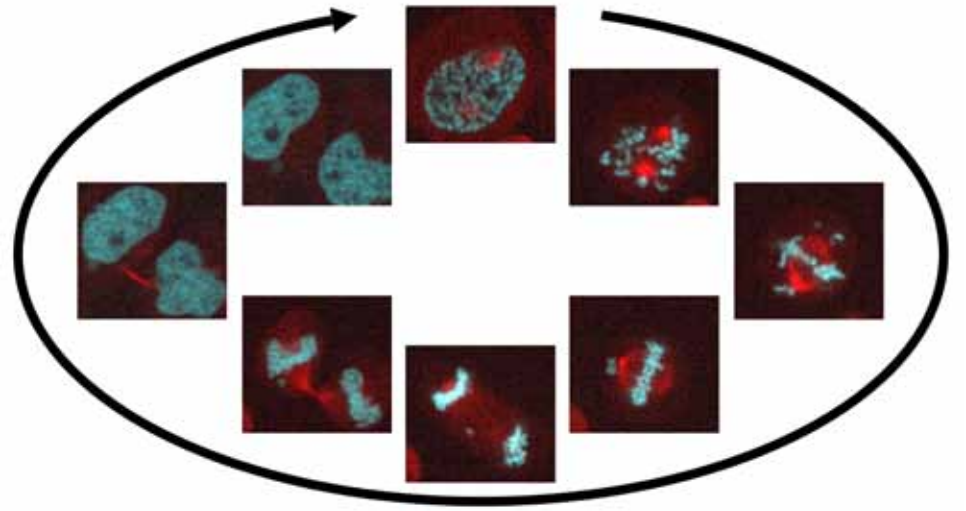


Figure 1. Possible mechanism of SHM.
AID is recruited to an *Ig* promoter (i), where it deaminates a cytosine residue in single-stranded DNA of a transcription bubble (ii). This gives rise to a U/G mispair in duplex DNA once the bubble has moved on (iii). This mispair may be detected by MutSα, but cannot be processed by MMR because it has no nicks in the vicinity. MutSα will therefore slide off and the uracil will be made available for processing by BER. If BER were to be interrupted immediately after the action of UNG2, the uncleaved AP-site might persist until DNA replication, where its by-pass by REV1 would give rise to mutations at C/G sites (iii). This situation changes once a second cytosine deamination takes place in the moving transcription bubble (iv). Should a partially-processed deamination site lie within ~1kb (the maximum distance between a mismatch and a nick), the MutSα sliding clamp activated by the newly-formed U/G (v) may interrupt the BER process at the distal uracil. If MutSα were to encounter an AP-site cleaved either by APE1 or MRE11, it might load EXO1 (vi) and the subsequent strand degradation would give rise to a single-stranded region spanning the distance between the first deamination site and ~150 nucleotides past the second one (vii). This gap would normally be filled-in by the replicative polymerase-η in an error-free manner. However, because the polymerase processivity factor PCNA in activated B-cells needs to be ubiquitylated (vii) in order for SHM to occur, and because ubiquitylated PCNA has a high affinity for the error-prone polymerase-η, it is possible that the ubiquitylated PCNA will recruit polymerase-η to a subset of the MMR repair patches (viii). Gap-filling by this error-prone enzyme (ix), which is known to introduce non-complementary nucleotides (N) opposite Ts, would give rise to mutations at A/T base pairs.

containing more than one uracil, such as would arise after the processive action of AID. This interference would give rise to atypically long repair tracts, which might be filled-in by error-prone polymerases (Figure 1).
We are currently attempting to obtain mechanistic insights into the CSR process by setting-up an *in vitro* system capable of mimicking the AID-triggered *in vivo* recombination events.

Figure 2. Time-lapse confocal live-cell images of cells stably-expressing mRFP-tagged histone H2B (shown in cyan) and GFP-tagged α-tubulin (shown in red), in which RuvBL1 was knocked down by siRNA. This sequence of images shows a chromosome mis-aligned during metaphase and anaphase. Chromosomal instability and mis-segregation are key characteristics of cancer cells.



The role of the RuvBL1 helicase in genomic instability

Dennis Castor

We identified RuvBL1 as an interaction partner of MLH1. The RuvBL1/RuvBL2 heterodimer has been indirectly implicated in many processes of DNA metabolism and we were interested to learn which of these processes requires its interaction with the MMR system. Surprisingly, indirect immunofluorescence studies showed that whereas RuvBL1/RuvBL2 the heterodimer was predominantly nuclear, RuvBL1 localised to the midbody in late telophase in the form of two well-defined foci. RuvBL2 was, in contrast, located exactly in between, which showed that the two subunits of the heterodimer were no longer interacting. We could subsequently show that RuvBL1 possessed consensus sites for polo-like kinase 1 (Plk1), a known regulator of mitosis. PLK1 was then shown to co-localise with RuvBL1 to the midbody and to phosphorylate RuvBL1 both *in vitro* and *in vivo*. We were also able to identify the phosphorylation site (in collaboration with Stefano Ferrari). Phospho-specific antibodies showed that the phosphorylated RuvBL1 localised to the centrosomes. RuvBL1 appears to play a critical role in chromosomal segregation, as its downregulation with RNAi results in an increased number of lagging chromosomes and genomic instability. The role of RuvBL1 in the maintenance of genomic stability requires its ATPase, as expression of a RuvBL1variant mutated in the ATP binding site resulted in cell death.

MMR and interstrand cross-link repair

Svenja Kaden, Katja Kratz, Barbara Schöpf

Using human cell lines stably expressing tagged MMR proteins, we characterised the interactome of MLH1 and PMS2 by proteomic analysis. The strongest interactors were FANCJ and an as-yet uncharacterised protein that we named REND1. The former protein is a helicase involved in the processing of interstrand cross-links (ICLs), and the *Fanconi anemia* pathway. This was very puzzling, given that MMR-deficient cells do not generally display different sensitivities to cross-linking agents such as cisplatin and mitomycin C than MMR-proficient cells and, conversely, cells defective in ICL processing are not known to have a mutator phenotype. Interestingly, REND1 turned out to be an exo/endonuclease, which preferentially cleaves 5′ flaps and D-loops. Knock-down of REND1 by siRNA sensitized cells to killing by cisplatin and MMC, which implied that this protein is also involved in ICL processing. We are currently attempting to find out which step of ICL repair requires REND1 (Figure 3) and also the biological relevance and/or importance of the binding of ICL-processing enzymes to MLH1 and PMS2.

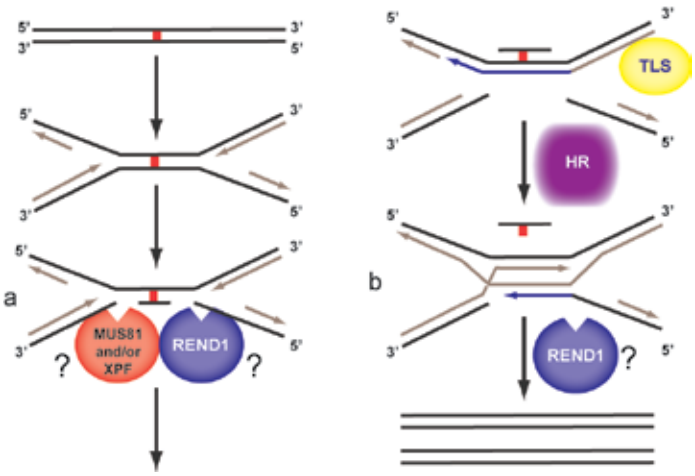


Figure 3. Possible roles of REND1 in ICL repair.
In the model proposed by Walter and colleagues (Raschle et al., Cell 2008), ICL processing takes place after two replication forks travelling in opposite directions converge on the cross-link. The leading strand template of one of the blocked forks could be incised by one of the 3′-flap specific endonucleases (MUS81-EME1 and/or XPF-ERCC1) and the lagging strand template of the second fork could be incised by REND1. This would create a double DSB and concurrently release an oligonucleotide fragment that is still covalently cross-linked to one of the template strands (structure a), but that is a substrate for TLS polymerases. An alternative is that REND1 cleaves the D-loop arising after HR-mediated repair of the DSBs at the blocked replication fork (structure b).

MMR and chromatin assembly

Barbara Schöpf (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 inhibits 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.

These projects are financed by the Bonizzi-Theler Fondation, the European Community, UBS AG and the Swiss National Science Foundation.

Massimo Lopes

Towards the structural visualization of genome instability during DNA replication

Our research takes advantage of molecular biology, cell biology and structural approaches to study DNA replication stress and its contribution to genome instability. We aim to understand the mechanistic basis of genome rearrangements arising during perturbed DNA replication, leading to cancer, as well as different neurological human syndromes.

Postdoc
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PhD students
Cindy Follonier
Arnab Ray Chaudhuri

Technician
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DNA replication represents a crucial, but potentially dangerous process that cells have to complete in order to undergo cellular division. A failure to properly maintain the integrity of replicating chromosomes inevitably leads to genome instability, which is an early event in tumorigenesis. Remarkably, most of the common anti-cancer drugs also interfere with the mechanism of DNA replication, relying on the generic observation that cancer cells, intrinsically deficient for DNA repair and cell cycle checkpoints, are sensitized to DNA damaging agents. Despite the importance of replication stress in both cancer onset and therapy, little structural and genetic information is available on the underlying molecular mechanisms.
During the first years of my scientific career I combined genetics and structural techniques (bi-dimensional DNA gel electrophoresis, electron microscopy) to investigate yeast DNA replication stress. In 2007 I established my independent research group AT the IMCR as Swiss National Science Foundation (SNF) Professor. We have expanded the portfolio of available techniques (DNA fiber spreading, DNA combing, Pulse Field Gel Electrophoresis) to investigate DNA replication stress in different eukaryotic systems (yeast, mammalian cell culture, Xenopus egg extracts) and to approach new biological questions related to genome instability, tumorigenesis and cancer therapy.

All the projects described below have been funded by the SNF-professorship grant and by the Einrichtungskredit of the University of Zurich.

Structural insights into oncogene-induced DNA replication stress

Kai Neelsen

Tumorigenesis is a multi-step process, driven by the accumulation of multiple genetic alterations. Their accumulation is in most cases accelerated by an increase in genomic instability, i.e. in the frequency of mutations and chromosomal rearrangements, which is therefore one of the earliest events in malignant conversion. A number of recent high-impact studies provided compelling evidence for this notion, demonstrating strong activation of the DNA damage response (DDR) in pre-invasive tumour stages and even in pre-malignant lesions of various organs. DNA damage checkpoints maintain the integrity of the genome by coordinating DNA repair and cell cycle progression in response to genotoxic insult or, if the damage proves too severe, triggering senescence or apoptosis, in order to minimise the risk of tumour development. These data establish the DNA damage checkpoint as an important anti-cancer barrier that helps to prevent tumourigenesis at its earliest stages.
The structures and events that trigger the DDR under these conditions are elusive. In the aforementioned studies, checkpoint activation has been attributed to unspecified alterations of bulk DNA replication. Indeed, the DNA damage response can be recapitulated in vitro by overexpression of various well-characterised oncogenes that are involved in the regulation of DNA replication, e.g. Cyclin E, E2F1, Cdc25A, c-Myc, mos, cdc6 and a constitutively active H-Ras mutant. Overexpression of these proteins lead to deregulation of DNA replication, either by activation of dormant origins or by unscheduled initiation of replication.
If "replication stress" is the trigger for the DNA damage checkpoint response to the expression of these oncogenes, what is the nature of the DNA structures generated during unscheduled and excessive DNA replication? How do these structures give rise to the DNA breaks that activate the DNA damage response and presumably lead to the chromosomal rearrangements found in oncogene-expressing cells?
Combining classical cellular and molecular biology assays (flow cytometry, pulse field electrophoresis, western blot for checkpoint factors) with single molecule approaches on replication intermediates (DNA fiber analysis, electron microscopy), our current investigations on oncogene-induced DNA replication stress suggest that replication forks experience elongation problems early after oncogene expression, whereas double strand breaks (DSB) become evident, both physically and through checkpoint activation, only later (Figure 1). These observations have been instrumental to design and perform electron microscopy experiments, by which we are currently asking which structural abnormalities at DNA replication intermediates precede and possibly determine DNA breakage early after oncogene overexpression in early tumorigenesis.

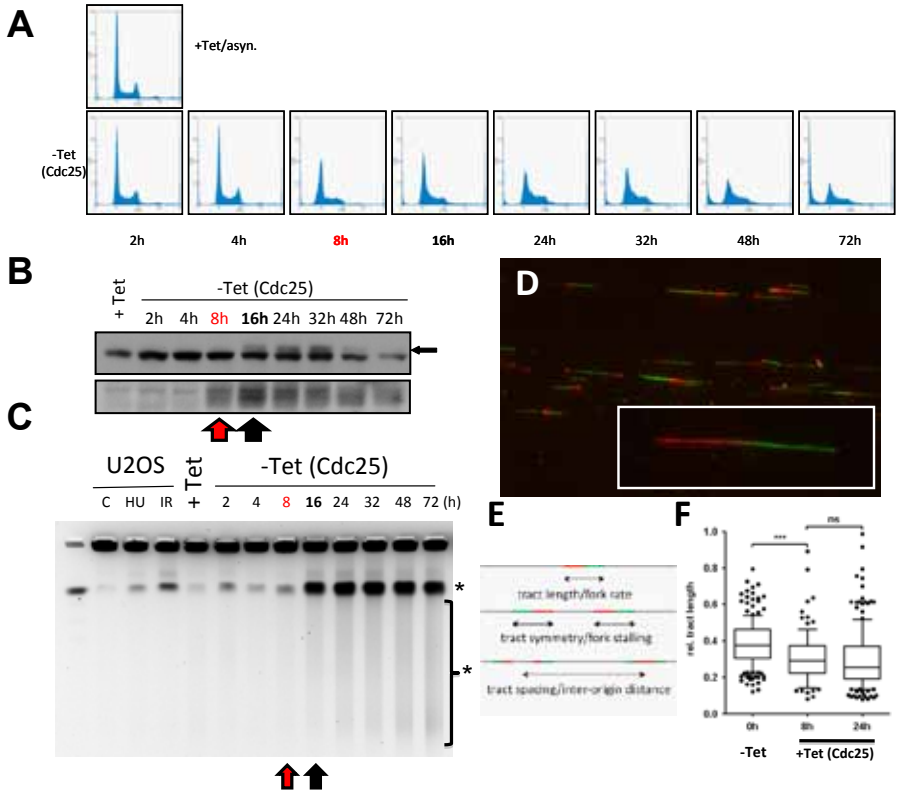

Figure 1. DNA replication stress precedes double strand break formation upon oncogene overexpression.

A) FACS analysis of Propidium Iodide (DNA) stained U2OS cells, overexpressing (O.P.) Cdc25A upon removal of Tetracycline from the culture medium. 8h after Tet-removal cells accumulate in S phase, while late time points show sub-G1 content indicative of apoptosis.

B) Western blot showing, upon Cdc25A O.P., the activation of the DNA damage response by the phosphorylation status of central checkpoint kinases Chk1 (by phosphospecific antibody) and Chk2 (by appearance of a retarded subpopulation of total Chk2 protein). ATR-mediated Chk1 activation signals the presence of DNA replication stress, while ATM-mediated Chk2 activation detects the presence of double strand breaks (DSB).

C) Physical detection of DSB upon Cdc25A O.P. by Pulse Field Gel Electrophoresis (PFGE). Cells are collected at different time points and embedded in agarose plugs, where mild conditions of DNA extraction limit the amount of breakage due to the procedure. Cdc25A O.P. leads at later time points to the massive induction of DSB (visible as DNA fragments entering the PFGE gel, *), well above the levels detected in control U2OS cells after nucleotide depletion (HU) or ionizing radiation (IR). Note that Chk1 phosphorylation signals problems at DNA replication fork already 8h after Tet removal (when cells show by FACS accumulation in S phase), while DSB are only detected (Chk2 phosphorylation, broken molecules in PFGE) after at least 16h of Cdc25A O.P., suggesting that DNA replication stress precedes and presumably triggers DSB formation.

D) Example of fluorescent read-out from a DNA fiber spreading experiment. Cells are labeled in vivo with consecutive pulses (20 min) of two different halogenated nucleotides (CldU and IdU) . Upon careful DNA extraction and uniform genomic DNA stretching over a silanized glass surface, replicated tracts are visualized by immunodetection (red and green, respectively) of DNA stretches where the two modified nulceotides have been incorporated.

E) Types of analysis and biological information that can be extracted by measuring length and distribution of the labeled DNA tracts in a standard DNA fiber spreading experiment.

F) Fork rate analysis upon Cdc25A O.P.: over 200 tracts are measured (length) and plotted. Length distribution reflects fork speed during the 20min labeling period. Cdc25A induces a striking reduction in fork speed (ca.30% slower), detectable already at time points after Tet-removal (8h), where no DSB can be detected directly or indirectly.

Uncovering the structural determinants of DNA replication stress induced by topoisomerase inhibition

Arnab Ray Chaudhuri (collaboration with Dr. V. Costanzo - Cancer Research UK and Prof. M. Foiani, IFOM, Milano, Italy)

Topoisomerase 1 (Top1) activity needs to be tightly regulated in every unperturbed cell cycle to ensure proper DNA replication fork progression and avoid DNA damage. Top1 can be trapped by specific inhibitors, leading to interference with DNA replication and transcription and resulting in potent cytotoxicity in proliferating and cancer cells. While the first natural Top1-inhibitor (Camptothecin, CPT) was identified more than 30 years ago, recent water-soluble derivatives (topotecan and irinotecan) have been approved by US Food and Drug Administration and are now frequently used in the treatment of ovarian, lung and colorectal cancer. A deeper understanding of the mechanisms by which Top1-inhibition interferes with DNA replication is therefore required to elucidate how potent anticancer drugs exert their cytotoxic effect on tumour cells. It would also help to identify additional cellular factors implicated in these mechanisms, which would represent promising drugable targets for combinational therapies.

Although replication-induced DSBs 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. Further structural investigation was therefore required to gain mechanistic insight into genomic instability induced by Top1 inhibition in normal and cancer cells.

We have investigated replication fork architecture and progression by a variety of structural and biochemical techniques, as well as by single molecule approaches applied to yeast, mammalian cells and Xenopus egg extracts, treated with Topl-inhibitors Camptothecin or Topotecan. Our observations suggest that distinct alterations of replication fork progression and structure - possibly resulting from accumulation of topological stress - precede DSB formation, challenging the current model of action of these drugs. i.e. replication fork run-off at DNA nicks (Figure 2).

By different genetic and biochemical tools (yeast mutants, siRNA in mammalian cells, immunodepletion in Xenopus extracts) we now plan to test the contribution of specific cellular factors likely to play a role in these mechanisms, with particular emphasis on the checkpoint control of fork stability and on the coordination of chromatin dynamics with DNA replication fork progression.

Structural analysis of DNA replication across unstable repetitive sequences

Cindy Follonier (collaboration with Prof. T. Mori - Nara Medical School, Japan)

The eukaryotic genome is not uniformly unstable. A large number of gross chromosomal rearrangements associated with cancer (such as translocations) have been mapped to specific genomic loci where the DNA can form unconventional (non-B) structures. In particular, trinucleotide repeats (TNR) have attracted enormous interest as model systems for studying genome instability problems related to DNA replication. A growing number of human neurological hereditary diseases, among which Huntington disease, Friedreich's Ataxia and fragile-X are the most prominent, have been associated with TNR expansion at various genomic loci. The TNR loci are typically polymorphic, such that the repeat lengths vary among individuals. This length variation is a sign of genomic instability, which increases with a growing number of repeats. Expansions of so-called "pre-mutation alleles" beyond a given threshold are associated with disease onset. A large body of evidence suggests that these events are associated with DNA replication "slippage" events. In bacterial, yeast and mammalian cells, replication forks have been shown to stall opposite TNR repeats. A common feature of all models proposed to account for these observations is the possibility that long TNR sequences form non-B DNA structures such as hairpins, slipped DNA structures, triplexes, or "sticky" DNA. Indeed, such structures 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 pre-mutation and disease alleles of the corresponding disease. Nonetheless, compelling evidence on which structures indeed form in living cells and contribute to TNR instability during DNA replication is still missing.

Taking advantage of TNR-containing plasmids, bi-dimensional DNA electrophoresis and electron microscopy in combination with in vivo-psoralen crosslinking, we are currently characterizing abnormal DNA structures - such as triplex DNA (Figure 3, A-B) and others - arising specifically at expanded GAA repeats during in vivo mammalian DNA replication. We are starting to test the role of candidate mammalian factors in the formation/resolution of these structures and, more generally, in the stability of repetitive tracts during replication. By large-scale molecular biological approaches, we also 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 specific antibody (Figure 3, C-E), and we are currently testing it in *in vivo* immuno-fluorescence experiments, as a novel diagnostic tool for Friedreich's Ataxia. More generally, this antibody may provide us with a simple read-out for genomic instability arising at replicating chromosomes. As a long-term goal, we aim to exploit this tool in genome-wide screens in mammalian cells, in an attempt to identify novel mammalian factors actively preventing genome instability during DNA replication.

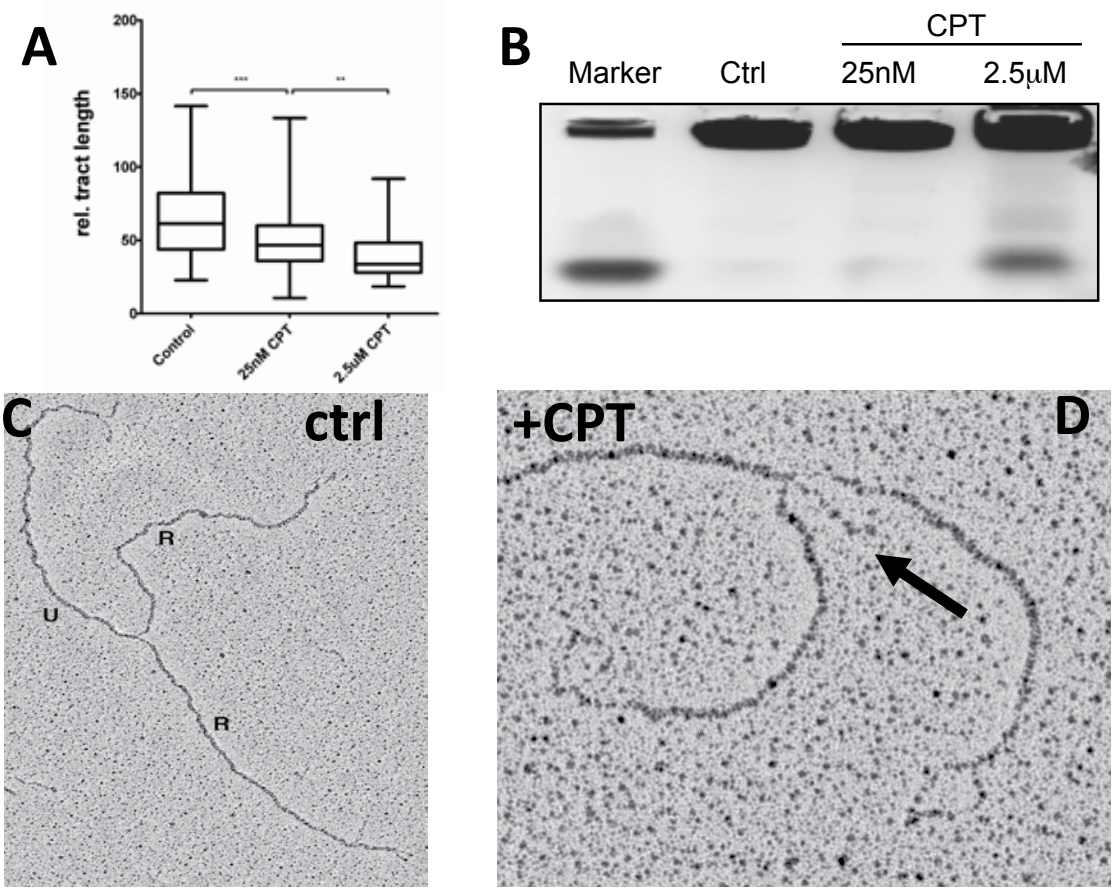

Figure 2. Topoisomerase-I inhibitors affect replication fork progression and structure even in the absence of detectable double strand breaks.
A) Fork rate analysis upon CPT treatment. Similarly to Cdc25 O.P. (Figure 1), CPT treatments lead to a highly significant (***) decrease in fork rate, suggesting that most forks experience problems replicating the template in presence of CPT, even at the lowest dose.
B) Pulse Field Gel Electrophoresis (PFGE) detection of DSB in U2OS treated with different CPT concentrations by. Higher CPT doses induce as expected DNA breakage (visible as DNA fragments entering the PFGE gel, *), but lower CPT doses - albeit sufficient to induce marked defects in fork progression and structure (Figure 2A and 2D) - do not lead to detectable levels of DSB.
C) An example of standard replication intermediate visualized by in vivo psoralen crosslinking, coupled to Transmission Electron Microscopy. U, unreplicated, parental duplex, R, replicated daughter duplexes.
D) An example of abnormal replication intermediate detected upon CPT treatments. The presence of a fourth arm at DNA replication forks (fork reversal) is a pathological phenomenon, probably resulting from the accumulation of topological stress induced by Top1 inhibition.

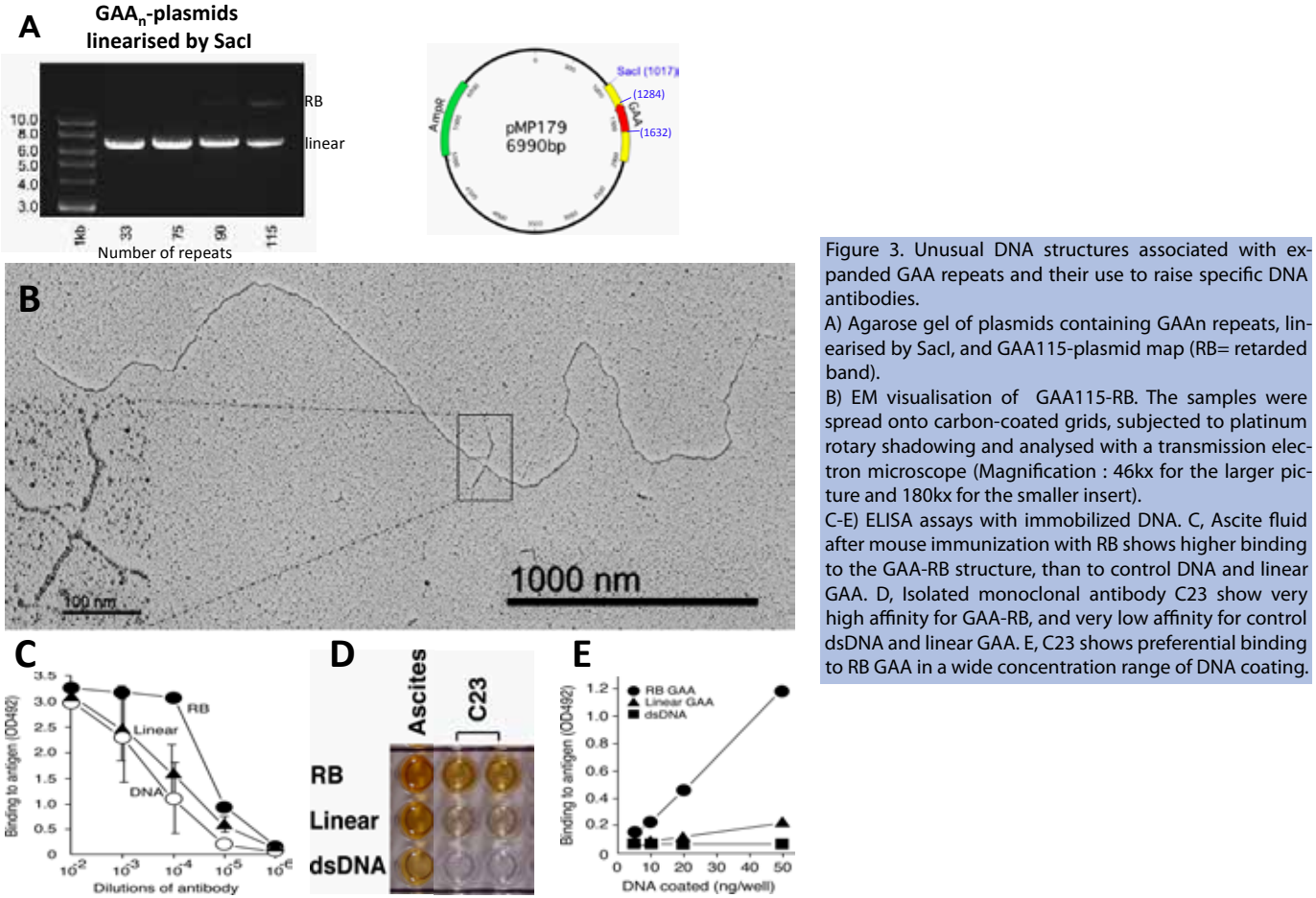

Figure 3. Unusual DNA structures associated with expanded GAA repeats and their use to raise specific DNA antibodies.
A) Agarose gel of plasmids containing GAAn repeats, linearised by SacI, and GAA115-plasmid map (RB= retarded band).
B) EM visualisation of GAA115-RB. The samples were spread onto carbon-coated grids, subjected to platinum rotary shadowing and analysed with a transmission electron microscope (Magnification : 46kx for the larger picture and 180kx for the smaller insert).
C-E) ELISA assays with immobilized DNA. C, Ascite fluid after mouse immunization with RB shows higher binding to the GAA-RB structure, than to control DNA and linear GAA. D, Isolated monoclonal antibody C23 show very high affinity for GAA-RB, and very low affinity for control dsDNA and linear GAA. E, C23 shows preferential binding to RB GAA in a wide concentration range of DNA coating.

Main collaborative studies

In collaboration with Dr. V. Costanzo (Cancer Research, UK) we have used the Xenopus egg extract system to investigate the molecular consequences of defects in the central recombination factor Rad51. Our data have revealed an unsuspected role for this DNA repair protein in the DNA replication process, even in the absence of exogenous DNA damage.

In collaboration with Prof. M. Muzi-Falconi (University of Milan, IT), we have identified yeast Exonuclease I as the cellular factor responsible for DNA damage checkpoint activation in response to UV damage; short ssDNA gaps generated by Nucleotide Excision Repair are frequently attacked by this nuclease to generate sufficient ssDNA for checkpoint activation.

In collaboration with the group of Dr. S. Ferrari within the IMCR we have uncovered a role for yeast 14-3-3 proteins in protecting stalled DNA replication forks from the nucleolytic processing mediated by Exonuclease I.

We are actively collaborating with the group of Prof. A. Müller within the IMCR to investigate whether Helicobacter pylori infection induced tumorigenesis is accompanied by DNA damage accumulation and DNA damage responses, similarly to the phenotypes induced by oncogene overexpresssion (Figure 1).

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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PhD Students
Elisa Cattaneo
Amit Tiwari

Technician
Ritva Haider

The genetic abnormalities associated with the adenoma-carcinoma sequence of tumor development in the human large intestine (colon) provided us with a basic model that has been progressively enriched with new insights, but it is now clear that colorectal carcinogenesis is a highly complex and heterogeneous process in which distinct tumor subsets evolve along different molecular pathways. The subset of colon cancers associated with defective DNA mismatch repair (MMR) was originally described in 1993. These tumors, which account for about 15% of all colorectal cancers, are characterized by alterations in the MSH2, MSH6, MLH1, and PMS2 genes, which are involved in the repair of DNA base-base mispairs and strand misalignments that occur during DNA replication. As a consequence of unrepaired strand misalignments, MMR(-) tumors show high-level microsatellite instability (MSI-H), a widespread genetic instability involving mono- and di-nucleotide repeats. Compared with their MMR-proficient counterparts, MMR(-) tumors are also characterized by a conspicuous lymphocytic infiltration and a better prognosis. Finally, they are resistant to the cytotoxic effects of alkylating agents and appear to be less responsive to standard fluorouracil-based adjuvant chemotherapy, so alternative forms of treatment are mandatory.

We are attempting to identify molecular markers of colon cancer formation and progression, with particular emphasis on those specific to MMR(-) tumors. To this aim, several techniques of molecular biology, biochemistry and cell biology are used, along with high-throughput screening procedures such as microarray analysis of the transcriptome of tumor cells. The basic research performed in this laboratory is strictly connected to the clinics, in particular to the work of pathologists, gastroenterologists and oncologists of the University of Zurich, the Triemli hospital of Zurich and other clinical centers.

Transcriptomic analysis of colorectal precancerous lesions and carcinomas

Elisa Cattaneo, Mirco Menigatti, Amit Tiwari

In collaboration with Endre Laczko, bioinformatician at the Functional Genomic Center Zurich

This program is based on the comparative analysis of transcriptomes of different precancerous lesions or malignant tumors of the colorectum and the normal colorectal mucosa. The tissues are collected in different hospitals and the transcript levels are detected with Affymetrix microarrays, which include in situ synthesized oligonucleotides representing the entire human genome. Hierarchical clustering and other analyses of the gene expression data effectively segregate the different categories of tissues being studied (see an example in Figure 1) and lead to the identification of hundreds of genes whose differential expression in these tissues is expected to shed light on the molecular processes involved in colon carcinogenesis. We are focusing on changes of the transcript level of transcription factors and Wnt target genes, and on changes caused by epigenetic transcriptional silencing. Thus far, these studies have yielded several interesting results, such as the overexpression of 4-1BBL -a crucial gene in the anti-tumor immune response- in MMR(-) tumor cells (di Pietro M. et al. Gastroenterology 2005, 129:1047-1059), the characterization of the intestinal Wnt signature gene set (Van der Flier LG. et.al. Gastroenterology 2007, 132: 628-632), the identification of KIAA1199 as a putative marker of colorectal adenomatous transformation (Sabates-Bellver J. et al., Molecular Cancer Research 2007; 5:1263-1275) and PTPRR as a novel marker of early epigenetic silencing (Menigatti M. et al., Molecular Cancer 2009, 8:124, doi:10.1186/1476-4598-8-124).

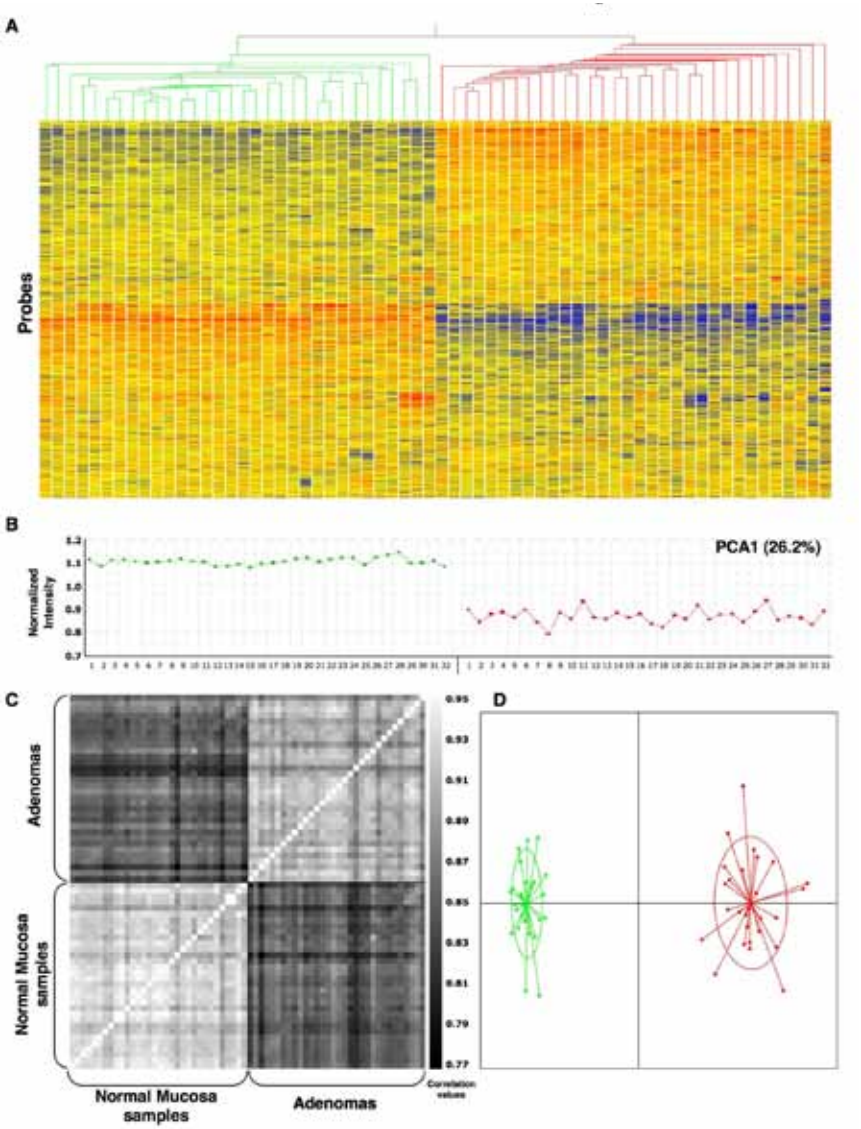


Figure 1. The transcriptome of colorectal adenomatous polyps differs significantly from that of the normal colorectal mucosa (unsupervised analyses of microarray data).
A) Hierarchical clustering analysis: 64 tissue samples represented on the x-axis include 32 normal mucosal samples (green branches) and 32 adenomas (red branches). Each probe plotted on the y-axis is color-coded to indicate the gene's level of expression relative to its median expression level across the entire tissue-sample set (blue: low; red: high).
B) Principal component analysis (PCA). Profile plot of the normalized first principal component (PCA-1) across the 64 specimens (green dots: normal mucosa; red dots: adenomas). The two tissue groups differ significantly in terms of PCA-1 ($p<0.0001$), which accounted for 26% of the total variance.
C) Correlation analysis. Tile-plot visualization of the pair-wise correlations of the samples. Correlation values are indicated on the gray-scale column (white > black: high > low). High correlation is observed among the samples within each group (upper right quadrant: adenomas; lower left quadrant: normal mucosa).
D) Correspondence analysis (CA) of mRNA log(intensity) values of expressed genes from 27 of the 32 tissue pairs (normal mucosa: green dots, adenoma: red dots). The areas delimited by the ellipses represent 95% of the estimated bi-normal distribution of the sample scores on the first and second CA axes. The map of the sample scores on the first two axes shows that CA efficiently discriminates between normal and adenoma samples.

Hereditary non-polyposis colon cancer (HNPCC) databank

Giancarlo Marra, Ritva Haider

Collaborators: Karl Heinimann and his group (University of Basel)

In one of our studies (Truninger K. et al., Gastroenterology 2005, 128:1160-1171), we set out to establish the true frequency of occurrence of colorectal cancers with defective mismatch repair (MMR). To this end, we analyzed 1048 unselected, consecutive colorectal cancers. Aberrant pattern of MMR protein expression was detected in 139/1048 colorectal cancers (13.3%). Loss of expression of MSH2, MSH6 or MLH1 was found in 1.4%, 0.5% and 9.8%, respectively (Figure 2A). As anticipated, lack of MLH1 expression was mostly associated with somatic silencing of the MLH1 promoter by cytosine methylation in sporadic (i.e. non familial) cases. However, about 3% of all colorectal cancers were MMR deficient and familial. These cases were included in a program of recruitment of individuals affected by colon cancer, whose clinical history showed evidence or suspicion of an inherited predisposition to this neoplasia. This program started eight years ago in collaboration with Dr. Karl Heinimann and his group (University of Basel). To date, we have recruited more than 300 index cases from the German-speaking cantons of Switzerland. Two hundred twelve of these have been thoroughly investigated by microsatellite instability analysis of their tumor DNA, immunostaining of tumors with antibodies against MMR proteins and extensive analysis of their germline DNA in search for mutations in the corresponding genes (Figure 2B). Irrespective of the selection criteria, a large fraction of our cases (139 out of 212 cases, 65.5%) showed a functional MMR (no MSI and normal immunostaining). These cases represent an important series to be exploited in search for novel genetic alterations predisposing their carriers to colon cancer.

Phenotype of subjects carrying heterozygous germline mutations of the MMR gene *PMS2*

Emanule Valtorta

Collaborator: Jan-Olaf Gebbers (Cantonal Hospital of Lucerne)

In our immunohistochemical screening (see above), a defect of a fourth MMR gene, PMS2, was found in 1.5% of consecutive colorectal cancers. Interestingly, the families of most patients had no obvious familial history of cancer. Immunohistochemical analysis revealed that PMS2 defects account for a considerable proportion of colorectal cancers, which have hitherto been classified as sporadic. DNA-based mutation detection methods have failed to identify these cancers in many studies, due to the interference of numerous PMS2 pseudogenes. To find out why the PMS2-deficient cases have a milder phenotype than patients/families with cancers carrying a defect in other mismatch repair genes, we are trying to characterize the germline alterations in the PMS2 locus of these patients. To date, we have found only few genetic alterations in the PMS2 gene (an insertion of one nucleotide in exon 11 in five patients, and a C to T transition in exon 6 in an additional case) by using classical mutational analysis tools. However, the numerous PMS2 pseudogenes on chromosome 7 (where PMS2 is located) may represent substrates for recombination events leading to deletion, inversion or other genomic alterations in the PMS2 locus that cannot be identified with these standard methods. Hampered by the presence of the numerous non-processed pseudogenes, even more sophisticated procedures (Multiplex Ligation-dependent Probe Amplification and Conversion of Diploidy to Haploidy) have not been able so far to pinpoint PMS2 genomic alterations, in our hands or in other labs. At present, we are developing alternative approaches to identify alterations in the PMS2 locus.

These projects are supported the Swiss National Science Foundation, the Zurich Cancer League, the Union Bank of Switzerland, the Sassella Stiftung, the Cancer League of Central Switzerland, and the Sophien-Stiftung.

A

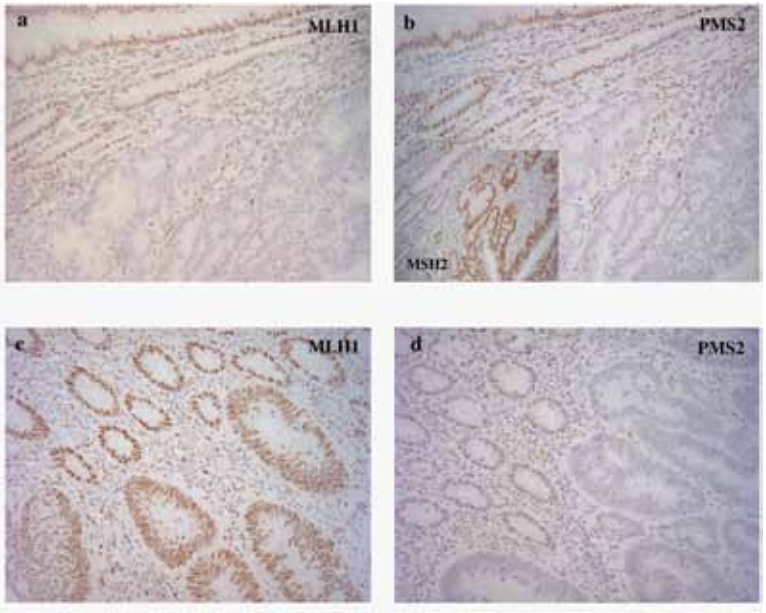
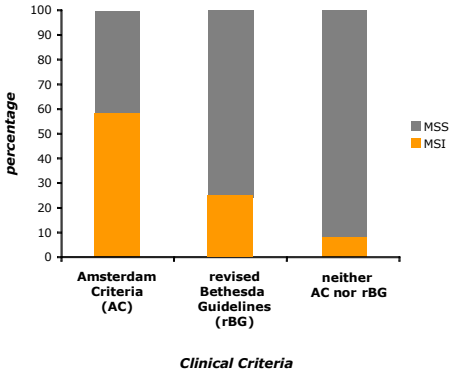


Figure 2. Colon cancers with defects in DNA mismatch repair.
A) Immunohistochemical staining of colorectal tumors for MMR proteins. Panels a and b: A tumor with a primary defect of the mismatch repair protein MLH1. a) MLH1 is absent from tumor tissue, but normal crypts (upper part of the picture) and proliferating stromal cells express this protein normally. b) The same tumor does not express PMS2, because this protein is unstable in the absence of MLH1. However, other MMR proteins are expressed normally, as shown for MSH2 in the inset. Panels c and d: Tumor with a primary defect of the mismatch repair protein PMS2. c) The dysplastic crypts on the right side of this tumor express MLH1 levels similar to the normal crypts on the left, however, d) the dysplastic crypts are deficient in PMS2.
B) Frequency of MSI-H in our series of 212 index cases suspected of genetic predisposition to colon cancer. Index cases are selected by using different clinical criteria such as the Amsterdam Criteria or the revised Bethesda Guidelines. Thirty-two percent of the cases (67) fulfilled the Amsterdam criteria, and 60% (39) showed microsatellite instability and loss of one of the MMR proteins. To date, a germline mutation in the corresponding genes was found in 32 of these 39 patients.

B



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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Pathogenesis of MALT lymphoma

Vanessa Craig

1. Gastric MALT lymphoma B-cells express polyreactive, somatically mutated immunoglobulins

Gastric B-cell lymphoma of mucosa-associated lymphoid tissue (MALT) arises against a background of chronic inflammation caused by persistent *Helicobacter pylori* infection. The clinical and histopathological features of the human tumor can be reproduced by *Helicobacter* infection of BALB/c mice. In this study, we have analyzed the antibody sequences and antigen specificity of a panel of murine and human MALT lymphoma-derived antibodies. We find that a majority of tumors in patients as well as experimentally infected mice are monoclonal. The tumor immunoglobulin heavy chain genes have undergone somatic hypermutation, and approximately half of all tumors show evidence of intraclonal variation and positive and/or negative selective pressure. Recombinantly expressed MALT lymphoma antibodies bind with intermediate affinity to various unrelated self and foreign antigens, including *Helicobacter* sonicate, IgG, DNA and stomach extract; antigen binding is blocked in a dose-dependent manner in competitive ELISAs. A strong bias towards the use of VH gene segments previously linked to auto- and/or polyreactive antibodies in B-cell malignancies or autoimmune pathologies supports the experimental finding of polyreactivity. Our results suggest that MALT lymphoma development may be facilitated by an array of local self and foreign antigens providing direct antigenic stimulation of the tumor cells via their B-cell receptor.

2. B-cell receptor signaling and CD40 ligand-independent T-cell help cooperate in *Helicobacter*-induced MALT lymphomagenesis

Using the murine model of the disease we demonstrate here that explanted tumor B-cells proliferate upon stimulation with the same panel of self and foreign antigens that are recognized by their surface antibodies. Tumor cell proliferation is strongly enhanced by the presence of intratumoral $CD4^+$ T-cells in a CD40/CD40L-independent manner. A large proportion of tumor-infiltrating $CD4^+$ T-cells are $CD25^+FoxP3^+$ Treg with highly suppressive activity, which are recruited by the tumor cells through secretion of the Treg-attracting chemokines CCL17 and CCL22. The depletion of $CD25^+$ cells was as efficient as $CD4^+$ T-cell depletion in blocking tumor growth *in vitro* and *in vivo*. In conclusion, our data suggest that B-cell receptor-derived signals cooperate with T-helper cell signals in driving the progression of MALT lymphoma, providing an explanation for the unique antigen dependence of this B-cell malignancy.

Pathogenesis of *Helicobacter* induced gastric cancer

Ayca Sayi, Esther Kohler, Isabelle Arnold, Mathias Oertli

1. The $CD4^+$ T-cell-mediated Interferon-g response to *Helicobacter* infection is essential for clearance and determines gastric cancer risk

Chronic infection with the bacterial pathogen *Helicobacter pylori* is a risk factor for the development of gastric cancer, yet remains asymptomatic in the majority of individuals. We report here that the C57Bl6 mouse model of experimental infection with the closely related *Helicobacter* felis recapitulates this wide range in host susceptibility. While the majority of infected animals develop premalignant lesions such as gastric atrophy, compensatory epithelial hyperplasia and intestinal metaplasia, a subset of mice is completely protected from preneoplasia. Protection is associated with a failure to mount an IFN-γ response to the infection and with a concomitant high *Helicobacter* burden. Using a vaccine model as well as primary infection and adoptive transfer models we demonstrate that IFN-γ secreted predominantly by $CD4^+CD25^-$ effector TH cells3, is essential for *Helicobacter* clearance, but at the same time mediates the formation of preneoplastic lesions. We further provide evidence that IFN-γ triggers a common transcriptional program in murine gastric epithelial cells *in vitro* and *in vivo*, and induces their preferential transformation to the hyperplastic phenotype. In summary, our data suggest a dual role for IFN-γ in *Helicobacter* pathogenesis that could be the basis for the differential susceptibility to *H. pylori*-induced gastric pathology in the human population.

2. TLR-2-activated B-cells suppress *Helicobacter*-induced preneoplastic gastric immunopathology by inducing T regulatory-1 cells

B-cells have counter-regulatory roles in autoimmune and chronic inflammatory conditions such as autoimmune encephalomyelitis and inflammatory bowel disease. We show here that B-cells can also negatively regulate adaptive immune responses to bacterial pathogens. Using mouse models of infection with *Helicobacter* felis, a close relative of the human gastrointestinal pathogen *H. pylor*i, we found that B-cells activated by *Helicobacter* TLR-2 ligands induce $CD4^+CD25^+IL^-10^+$ T regulatory-1-like cells *in vitro* and i*n vivo*. Tr-1 conversion depends on TCR signalling and a direct T-/B-interaction through CD40/CD40L and CD80/CD28. B-cell-induced Tr-1 cells acquire suppressive activity *in vitro* and suppress excessive gastric *Helicobacter*-associated immunopathology *in vivo*. Adoptive co-

transfer of MyD88-proficient B-cells and Tr-1 cells restores a normal gastric mucosal architecture in MyD88$^{-/-}$, TLR-2$^{-/-}$ and IL-10$^{-/-}$ mice in a manner that depends on T-cellular, but not B-cellular IL-10 production. Our findings describe a novel mechanism of Tr-1 cell generation and function in a clinically relevant disease model.

3. Tolerance rather than immunity protects from *Helicobacter* pylori-induced gastric preneoplasia

Chronic infection with the bacterial pathogen *Helicobacter pylori* causes gastric disorders ranging from chronic gastritis and gastroduodenal ulcers to adenocarcinoma. Only a subset of infected individuals will develop overt disease; the large majority remains asymptomatic despite lifelong high-level colonization. This study aims to mechanistically elucidate the differential susceptibility to *H. pylori* that is found both across and within populations. We have established a C57BL/6 mouse model of *H. pylori* infection with a strain that is capable of delivering the virulence factor CagA into host cells through the activity of a Cag-pathogenicity island-encoded type IV secretion system. Mice infected at 5-6 weeks of age with CagA^{+} *H. pylori* rapidly develop gastritis, gastric atrophy, epithelial hyperplasia and intestinal metaplasia in a type IV secretion system-dependent manner. In contrast, mice infected during the neonatal period with the same strain are protected from preneoplastic lesions. Their protection is due to the development of *H. pylori*-specific peripheral immunological tolerance, which requires TGF-beta signalling and is mediated by long-lived, inducible regulatory T-cells, and which efficiently controls the local CD4^{+} T-cell responses that trigger premalignant transformation. Tolerance to *H. pylori* develops in the neonatal period due to a strongly biased Treg to T-effector cell ratio, and is favoured by prolonged low-dose exposure to antigen. In conclusion, using a novel CagA^{+} *H. pylori* infection model, we report here that the development and maintenance of tolerance to *H. pylori* protects from gastric cancer precursor lesions. The age at initial infection may thus account for the differential susceptibility of infected individuals to *H. pylori*-associated disease manifestations.

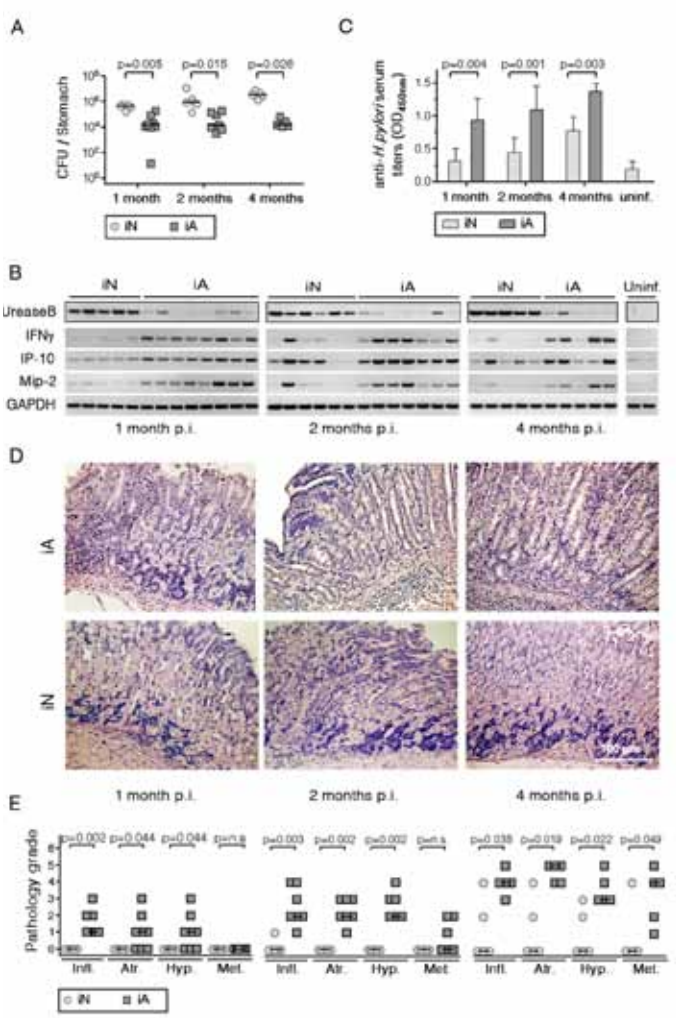


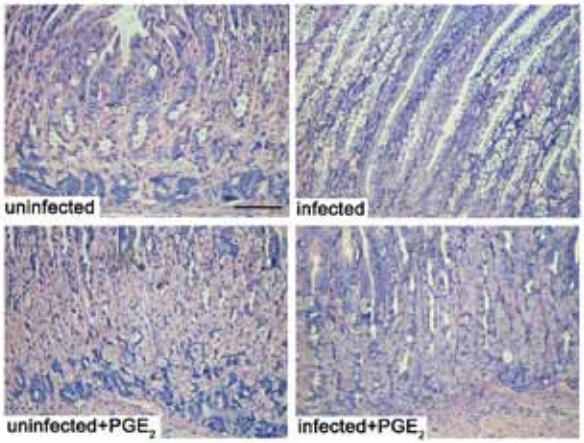
Figure 1. Neonatally infected mice fail to mount local and systemic immune responses to *H. pylori* infection and are protected from preneoplastic gastric pathology. (A-E) C57BL/6 mice were infected with *H. pylori* PMSS1 at either 7 days (infected as neonates, iN) or 5 weeks of age (infected as adults, iA) in groups of 5-8 animals and were sacrificed at 1, 2 and 4 months p.i. (A) CFU per stomach were determined for individual mice; horizontal bars indicate the medians for every group. (B) UreaseB-specific PCR was performed as an additional measure of colonization (uppermost panels). The gastric production of the indicated proinflammatory cytokines was evaluated by RT-PCR with GAPDH levels as controls. (C) Serum titers to H. pylori as determined by ELISA. The average titer of two uninfected mice is shown for comparison. (D, E) Gastric histopathology of neonatally and adult-infected mice. Representative micrographs are shown in D and histopathology scores are shown in E. Data are representative of 2-3 experiments per time point.

The role of COX-2, PGE_2 and PARP in *Helicobacter*-induced gastric cancer

Isabella Toller, Esther Kohler, Iris Hitzler

1. Prostaglandin E2 prevents *Helicobacter-i*nduced gastric preneoplasia and facilitates persistent infection in a mouse model

Persistent infection with the human pathogen *Helicobacter pylori* increases the risk of gastric cancer. In this study, we investigated the role of cyclooxygenase-2 (COX-2) and its main product, prostaglandin E2 (PGE_2), in the development of Helicobacter-induced gastritis and gastric cancer precursor lesions. We utilized mouse models of *Helicobacter*-induced gastric preneoplasia and vaccine-induced protection to study the effects of COX-2 inhibition and PGE_2 treatment on the induction of *Helicobacter*-specific immune responses and gastric premalignant immunopathology. COX-2 and PGE_2 are up-regulated upon *Helicobacter* infection in cultured epithelial cells and in the gastric mucosa of infected mice. Inhibition of COX-2 activity with Celecoxib significantly accelerated early preneoplasia; conversely, systemic administration of synthetic PGE_2 prevented the development of premalignant pathology and completely reversed pre-existing lesions by suppressing IFN-γ production in the infected stomachs. The protective effect of PGE_2 was accompanied by increased Helicobacter colonization in all models. All in vivo effects were attributed to immunosuppressive effects of PGE_2 on $CD4^+$ T-helper 1 (Th1) cells, which fail to migrate, proliferate and secrete cytokines when exposed to PGE_2 in vitro and in vivo. Tcell inhibition was found to be due to silencing of IL-2 gene transcription, and could be overcome by supplementation with recombinant IL-2 in vitro and in vivo. In conclusion, COX-2-dependent production of PGE_2 has an important immunomodulatory role during *Helicobacter* infection, preventing excessive local immune responses and the associated immunopathology by inhibiting the effector functions of pathogenic Th1 cells.



2. Inhibition of ADP-ribosylation prevents and cures *Helicobacter*-induced gastric preneoplasia

Gastric adenocarcinoma develops as a consequence of chronic inflammation of the stomach lining that is caused by persistent infection with the bacterium *Helicobacter pylori*. Gastric carcinogenesis progresses through a sequence of preneoplastic lesions that manifest histologically as atrophic gastritis, intestinal metaplasia and dysplasia. We show here in several preclinical models of *Helicobacter*-induced atrophic gastritis, epithelial hyperplasia and metaplasia that inhibition of ADP-ribosylation by the small molecule inhibitor PJ34 not only prevents the formation of gastric cancer precursor lesions, but also efficiently reverses pre-existing lesions. PJ34 exerts its chemopreventive and therapeutic effects by impairing *Helicobacter*-specific T-cell priming and TH1 polarization in the gut-draining mesenteric lymph nodes (MLN). The subsequent infiltration of pathogenic T-cells into the gastric mucosa and the ensuing gastric T-cell-driven immunopathology are prevented efficiently by PJ34. Our data indicate that PJ34 directly suppresses T-cell effector functions by blocking IFN-γ production of MLN T-cells *ex vivo*. Upon exposure to PJ34, purified T-cells failed to synthesize ADP-ribose polymers and to activate the transcription of genes encoding IFN-γ, IL-2 and the IL-2R α chain in response to stimuli such as CD3/CD28 crosslinking or PMA/ionomycin. The immunosuppressive and chemoprotective effects of PJ34 therefore result from impaired T-cell activation and TH1 polarization and lead to protection from preneoplastic gastric immunopathology. In conclusion, ADP-ribosylating enzymes constitute novel targets for the treatment of *Helicobacter*-associated gastric lesions predisposing infected individuals to gastric cancer and may also hold promise for the treatment of other T-cell-driven chronic inflammatory conditions and auto-immune pathologies.

Figure 2. PGE_2 treatment of *H.* felis infected $IL10^{-/-}$BL/6 mice inhibits chronic inflammation and epithelial pathology
$IL10^{-/-}$BL/6 mice were infected for one month with H. felis and were treated with PGE_2 starting on the day of infection (A). Representative Giemsa-stained sections and histopathology scores are shown in A and B; the quantification of PCNA-positive hyperplasia is depicted in C. Neutrophil infiltration was quantified by myeloperoxidase activity assay (averages of all mice per group are shown with standard deviations, D), and gastric IFN-γ expression was assessed by real time PCR (E; * not detectable).

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

Iris Hitzler

Gastric cancer is the third most commonly diagnosed cancer and the second most common cause of cancer-related deaths worldwide. Because of its late detection and dismal treatment options, the survival rates for gastric cancer patients are poor. Gastric cancer is caused in a vast majority of patients by chronic infection with the bacterial pathogen *Helicobacter pylori*. Therefore, vaccination against the bacterium is widely viewed to be the most promising strategy of gastric cancer prevention.

The development of a vaccine against *Helicobacter* has proven to be much more difficult than initially envisioned. None of the regimens that showed efficacy in preclinical models are suitable for human use, either because they utilize adjuvants that are toxic to humans, or because, in humans, they do not elicit the T-cell responses that are essential for protection. We have published previously that vaccination prevents mucosa-associated tissue (MALT) lymphoma, another gastric malignancy directly linked to Helicobacter infection. In the past two years, we have made an effort to better understand the mechanism of protection of the current gold standard *Helicobacter* vaccine in order to rationalize vaccine development against *H. pylori*.

We plan to extend the program to address the following issues, which we believe are key to the improvement of existing preclinical vaccine regimens, and to the adaptation of the vaccine for human use. First, we aim to better understand through which mechanisms and in which secondary lymphoid tissues protective T-cells are primed, and which effector mechanisms ultimately lead to the elimination of the bacteria. Second, we will compare various routes of vaccine administration, and replace the currently used mucosal adjuvant cholera toxin by other less toxic adjuvant formulations. One compound in particular, the CAF01 adjuvant provided to us by collaborators at the Danish Serum Institute, has shown promising efficacy against *Helicobacter* in preliminary studies. Finally, we have started to identify new protective *Helicobacter* antigens that could replace the crude whole cell sonicate that is currently the best option in preclinical models of immunization against *Helicobacter.*

These projects are funded by grants from the Swiss National Science Foundation, Oncosuisse, UBS Desiree und Nils Yde Foundation and by start-up funding of the University of Zurich awarded to Anne Müller.

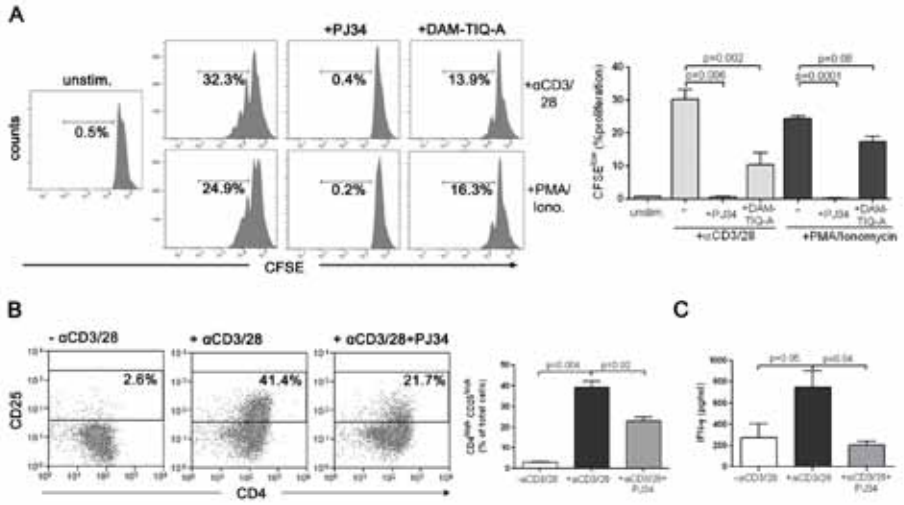


Figure 3. Inhibition of ADP-ribosylation prevents T-cell activation by CD3/CD28 crosslinking or treatment with PMA/ionomycin.

$CD4^+CD25^-$ cells were isolated from single cell preparations of the combined MLN of five mice. The cells were labeled with CFSE and stimulated for three days with αCD3/28-coated beads or PMA/ionomycin; 15μM PJ34 or 15μM DAM-TIQ-A was added where indicated. (A) CFSE dilution as a measure of proliferation was assessed flow cytometrically. Representative histograms (left panels) as well as the averages of triplicate cultures (right panel) are shown. (B) CD25 expression was assessed by surface staining. Representative dot plots and averages of triplicate cultures are shown. (C) The αCD3/CD28-induced secretion of IFN-γ into the culture supernatants was measured by ELISA for the same cultures as shown in B.

Alessandro A. Sartori

DNA double-strand break repair and cancer

Studying the causes and consequences of DNA damage is a major topic of research within cancer biology. DNA damage not only causes cancer but is also used as a means to cure cancers through radio- or chemotherapy and is also responsible for the side effects of these treatments. DNA double-strand breaks (DSBs) are the most cytotoxic DNA damage lesions induced by ionizing radiation and certain anti-cancer drugs, and appropriate responses to 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 DNA double-strand breaks (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, for example, post-translational modifications. 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. In our laboratory, we focus on the HR-mediated branch of DSB repair, mainly because its function is intimately linked to DNA replication. The elucidation of the interplay between these two fundamental cellular metabolic pathways is another topic of interest in our laboratory.

To preserve genomic integrity and survival, DSBs alarm the cellular DNA damage response machinery, a multifaceted response orchestrated by the ATM and ATR kinase signaling pathways in mammalian cells. 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 triggering ATR activation and repair by homologous recombination (HR). 5′ to 3′ resection of DSB ends, a process that is still not understood in great detail, has been shown to be dependent on both ATM and CDK (cyclin dependent kinase) activities, but the targets of these kinases that are involved in DNA resection have not yet been identified (Figures 1 and 2).

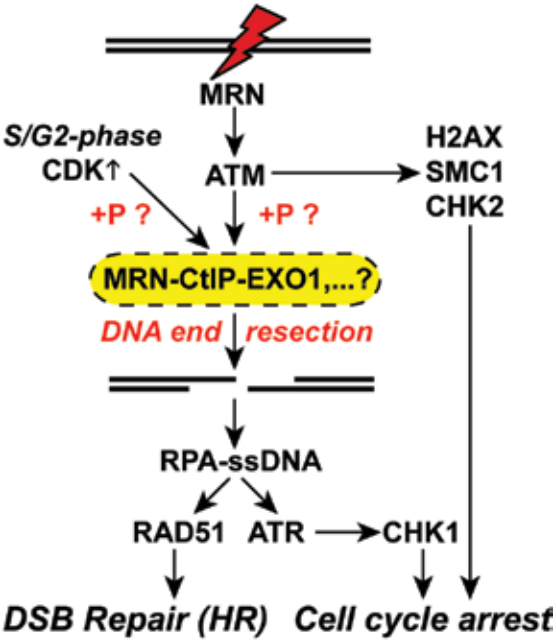
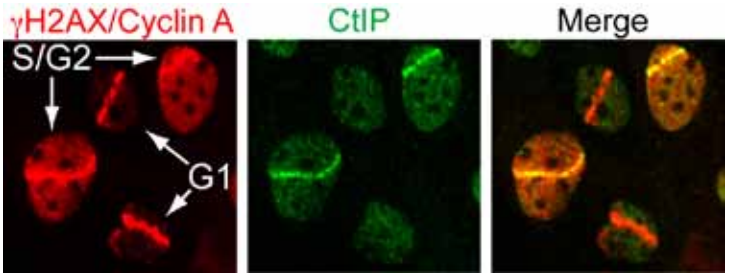


Figure 1. DSB end resection is a crucial step in both HR and checkpoint signaling.
DSBs are processed by CtIP, MRN and EXO1 to expose ssDNA tails that are immediately bound by RPA. The RPA-coated ssDNA recruits ATR, leading to the activation of downstream effectors such as Chk1 to initiate cell cycle arrest. In order to proceed with the repair by HR, RPA has to be replaced by RAD51 to form the presynaptic filament. DNA resection is known to be regulated by ATM- and CDK-dependent phosphorylation (+P) events, but the target proteins still remain to be identified.

Our projects are funded by University of Zurich and Swiss National Science Foundation.

A



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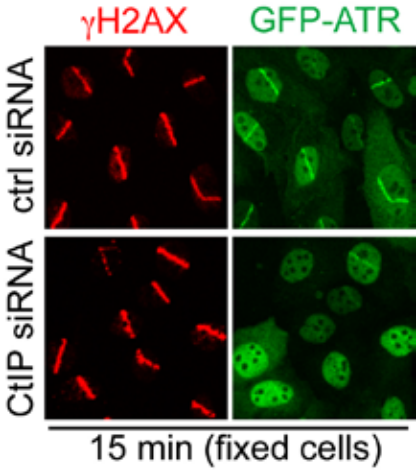
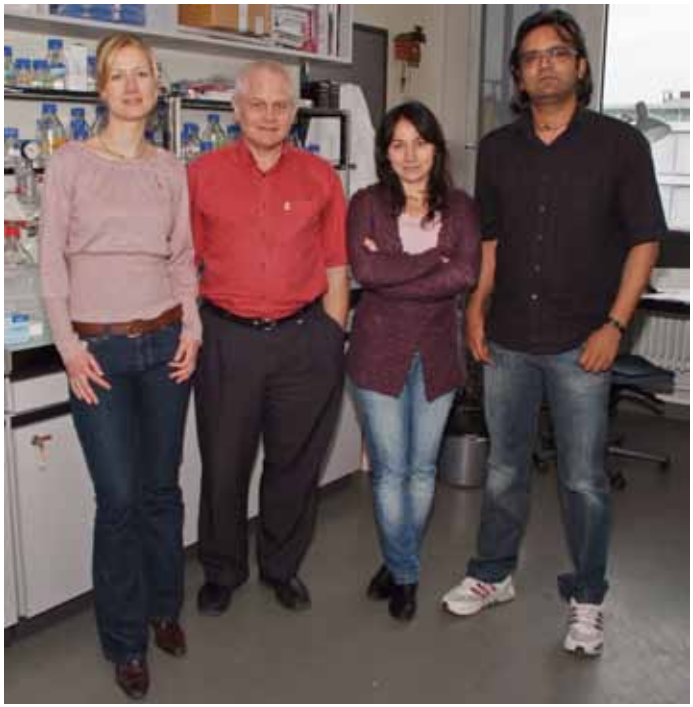


Figure 2. CtIP associates with sites of DNA damage in S/G2 phase and promotes ATR recruitment to DSBs.
A) CtIP recruitment to laser-induced DSBs occurs in S/G2 phase. Cells were stained for endogenous γH2AX, CtIP or cyclin A. All cells have local γH2AX signals but only S/G2-phase cells have pan-nuclear cyclin A staining.
B) CtIP depletion does not affect γH2AX formation. Cells were fixed 15 min after micro-irradiation and immunostained. Images to evaluate cellular responses to laser damage were acquired by Claudia Lukas (Institute of Cancer Biology and Centre for Genotoxic Stress Research, Copenhagen, Denmark). Sartori, A.A. et al. Nature 450, 509-514 (2007).

Reto Schwendener

Tumor-associated macrophages

Depletion of tumor-associated macrophages (TAMs) with bisphosphonate-liposomes leads to pronounced anti-angiogenic effects and growth inhibition of solid tumors. We investigate the potential of this new method by two ways, namely, 1) as tool to study basic biological and immunological effects in the tumor microenvironment and 2) as cancer therapy approach in mouse tumor models to contribute to a better understanding of the complex processes of tumor development, growth and metastatic dissemination.



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Sushil Kumar

PhD Students
Anne-Katrin Bonde
Sibel Mete

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 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. However, it is now acknowledged that tumors are largely recognized as self and lack strong antigens. Instead, they appear to have been selected to manipulate the host immune system to prevent rejection and to use it 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 findings, given that high densities of leukocytic infiltrations, most notably macrophages, correlate with poor prognosis of the diseases. TAMs are derived from circulating monocytes and are activated macrophages of the polarized type II (M2 macrophages, alternatively activated 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, which promote tumor angiogenesis and growth. Due to their tumorigenic role, TAMs have been proposed as potential therapeutic targets. Several therapeutic strategies have recently been designed or suggested to target different cells, including macrophages, in the tumor microenvironment. To study the role of TAMs, we apply a bisphosphonate-liposome mediated TAM depletion method in various slow- and fast growing subcutaneous and orthotopic mouse tumor and metastasis models. Tumor growth and formation of metastases are monitored by live imaging, immunohistochemistry, flow cytometry, confocal microscopy and by genomic and proteomic approaches. Additionally, we use in vitro co-culture models to identify nascent proteins translated in cancer and endothelial cells in response to macrophages.

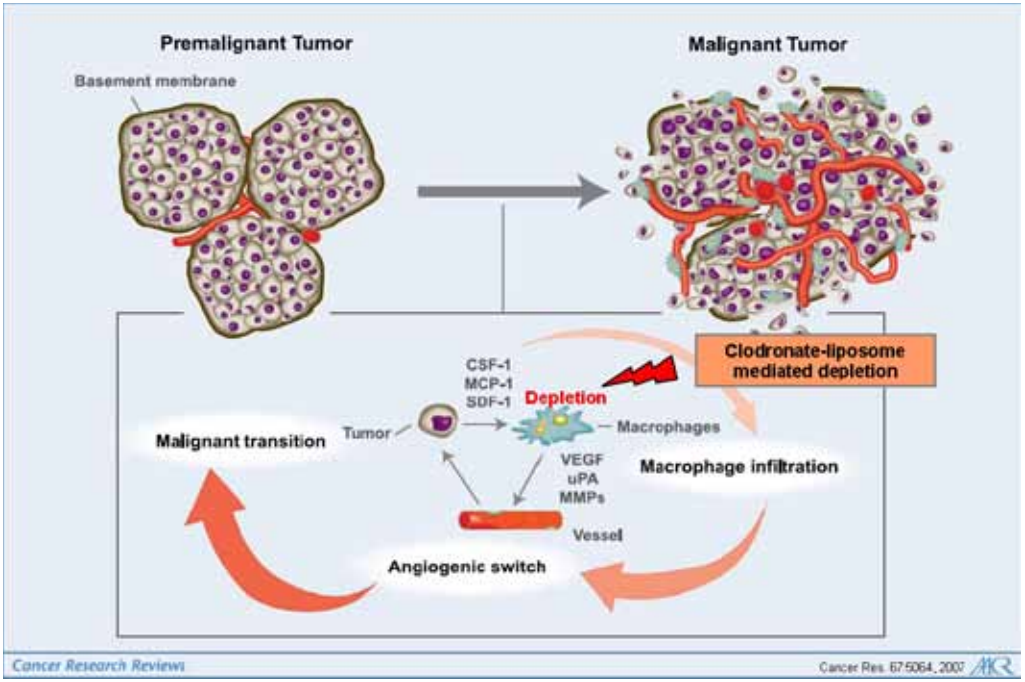

Figure 1. Macrophages promote tumor progression through stimulation of the angiogenic switch. Depletion with clodronate-liposomes inhibits angiogenesis and other tumorigenic processes.

Molecular characterization of cancer-macrophage interactions and screening of new drug targets in macrophages

Sushil Kumar, Sibel Mete

Many conventional tumor therapies are marred due to the genetic instability of cancer cells, which leads to drug resistance. Since macrophages are shown to promote tumor 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 macrophages and apply their inhibitors in liposomal formulations to specifically target macrophages, since some of these signaling pathways are tumor suppressive. To achieve this, we have established co-culture models of melanoma and macrophage cell lines, which will be used to screen the inhibitors of inflammatory signaling pathways. To validate the key molecules and drugs identified in both screens, TAM depletion in mice will be used, coupled with tissue microarray analysis. In addition, we will utilize the conditioned medium of cancer cells and macrophages to profile the secreted molecules from these cell types, which will add to our understanding of cancer-macrophage cross-talk.
The fulfillment of this project will have significant impact on our understanding of the tumor microenvironment and for the development of new therapies to fight against cancer.

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 macrophages to 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 tumor associated macrophages (TAMs) in 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 elucidated 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 a main regulator of the mesenchymal phenotype in F9-cells and murine mammary gland NMuMG-cells. Moreover,

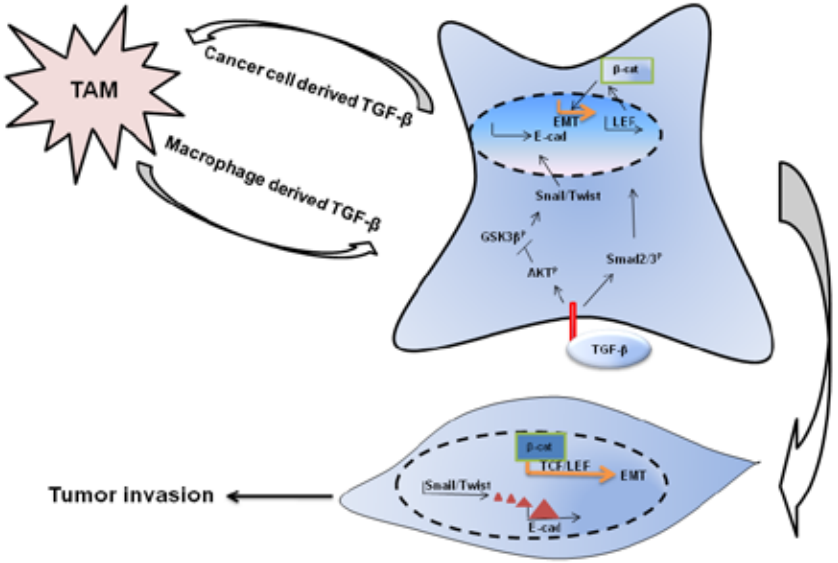

Figure 2. Schematic representation of the regulatory role of TAMs in epithelial to mesenchymal transition. We identified TAM derived TGF-β as a main regulator of the mesenchymal phenotype through activation of the β-catenin/TCF-LEF pathway.

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 a 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.

These projects are funded by the Swiss National Science Foundation (SNF) and Novartis.

Establishment of *in vivo* imaging methods and investigation of strategies by which tumor associated macrophages can be redirected towards tumor rejection

Sushil Kumar, Sibel Mete

To elucidate the roles of TAMs in metastatic dissemination, we established orthotopic murine melanoma models. Metastasizing B16 melanoma cells were orthotopically injected into the footpads of mice. Metastases are found at day 7 in the sentinel lymph nodes, followed by distant metastases in liver and lungs 3-4 weeks after tumor inoculation. In TAM-depleted mice, the growth rate of the primary tumors is reduced compared to untreated mice. To evaluate and visualize tumor growth and the rate of metastatic spread, the B16 cells were transfected with red fluorescent protein (mCherry-RFP) and Click Beetle luciferase constructs. Results obtained from the in vivo study will be validated and further elaborated by tissue whole mounts and immunohistochemistry and complemented by ex vivo and in vitro experiments, particularly focusing on TAM-specific chemokine/cytokine levels both at the primary tumor site and at the site of metastasis.

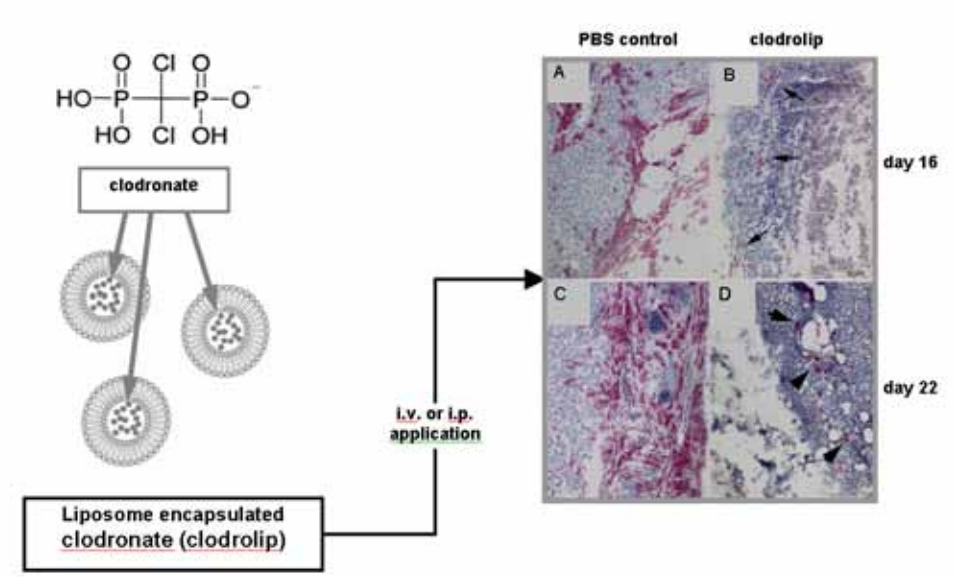

Figure 3. Clodronate-liposome mediated depletion of TAMs in the A673 rhabdomyosarcoma model. Scheme of clodronate encapsulation (left), and immunohistochemical staining with the macrophage specific F4/80 antibody (right, from Br. J. Cancer 95: 272, 2006).

Administrative, IT and Laboratory Support

This section of the Institute fulfils multiple roles. The administrators deal with personnel and financial matters, and co-ordinate the Cancer Network Zurich, 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.

Peter Binz
Cornelia Geiser
Patrick Greiner
Marianne Köpfler
Farah Mhamedi
Christoph Moser
Jana Rojickova
Malika Salah
Cornelia Schauz

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Dissertations

Radhakrishnan, K.
Biochemistry of Human RecQ DNA Helicases and their Role in DNA Repair

Schanz, S.
Involvement of mismatch repair proteins in activation-induced deaminase-mediated antibody diversification

Zheng, L.
The role of human RECQ5 helicase in the maintenance of genomic stability revealed by protein-protein interaction study

2009

Bijnsdorp, I V; Schwendener, R A; Schott, H; Fichtner, I; Smid, K; Laan, A C; Schott, S; Losekoot, N; Honeywell, R J; Peters, G J.
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Gastric MALT lymphoma B cells express polyreactive, somatically mutated immunoglobulins. Blood.

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Lopes, M.
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Schanz , S; Castor, D; Fischer, F; Jiricny, J.
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Toller, I M; Hitzler, I; Sayi, A; Mueller, A.
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Torroella-Kouri, M; Silvera, R; Rodriguez, D; Caso, R; Shatry, A; Opiela, S; Ilkovitch, D; Schwendener, R; Iragavarapu-Charyulu, V; Cardentey, Y; Strbo, N; Lopez, D M.
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Wanner, R M; Castor, D; Güthlein, C; Böttger, E C; Springer, B; Jiricny, J.
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Zheng, L; Kanagaraj, R; Mihaljevic, B; Schwendener, S; Sartori, A A; Gerrits, B; Shevelev, I; Janscak, P.
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Zumsteg, A; Baeriswyl, V; Imaizumi, N; Schwendener, R; Rüegg, C; Christofori, G.
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Habilitations

Marra, G
Understanding tumorigenesis of the large intestine: The cornerstone of effective cancer prevention strategies

Dissertations

Castor, D
Functional characterization of RuvB-like 1 during cell division

Cattaneo, E
The transcriptomic profiling of human colorectal precancerous lesions

Kehl, P
Inducible Gene Replacement of DNA Polymerase Delta

Schwendener, S
Functional characterization of the human RECQ5 protein

Collaborations

Academy of Sciences of the Czech Republic, Prague, Czech Republic
Beckman Research Institute of the City of Hope, USA
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Cancer Research UK, London Research Institute, UK
Cantonal Hospital Lucerne, Switzerland
Cantonal Hospital St. Gallen, Switzerland
Case Western Reserve University, Cleveland, Ohio, USA
Centre Europeen pour la Recherche en Biologie et Medecine, France
CRC laboratories, UK
Danish Cancer Society, Denmark
Duke University, USA
École Polytechnique Fédérale de Lausanne, Switzerland
Erasmus University, Rotterdam, The Netherlands
ETH Zurich, Switzerland
Flinders University, Adelaide, South Australia
Friedrich Miescher Institute, Basel, Switzerland
Hannover Medical School, Hannover, Germany
Harvard University, Cambridge, USA
Hospital Cremona, Italy
Hubrecht Institute, Utrecht, The Netherlands
ICGEB, Trieste, Italy
IFOM, Italy
IGBMC, Strasbourg, France
Imperial College London, UK
Indiana University School of Medicine
Institute of molecular genetics AS CR, Prague, Czech Republic
Institute of Veterinary Biochemistry, University of Zurich, Switzerland
Istituto Dermopatico dell'Immacolata, Rome, Italy
Johns Hopkins School of Medicine, Baltimore, USA
Justus-Liebig-University Giessen, Giessen, Germany
Korea Advanced Institute of Science and Technology, Republic of Korea
KuDOS Pharmaceutical Limited, UK
Kyoto University, Kyoto, Japan
Leiden University Medical Center, The Netherlands
Ludwig-Maximilians-University, Munich, Germany
Masaryk University, Brno, Czech Republic
Massachusetts Institute of Technology, Cambridge, USA
Max Planck Institute for Molecular Genetics, Berlin, Germany
Mount Sinai School of Medicine, New York, USA
Nara Medical University, Kashihara, Nara, Japan
National Cancer Institute, Frederick, MD, USA
National Institutes of Health (NIH), Bethesda, MD, USA
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Neurimmune Therapeutics AG, Zurich, Switzerland
Norwegian Univerity of Science and Technology, Norway
Novartis Pharmaceuticals, Basel, Switzerland
Paul-Ehrlich-Institute, Langen, Germany
Rockefeller University, New York, USA
Roskilde University, Roskilde, Denmark
Slovak Academy of Sciences, Bratislava, Slovakia
Stanford University, USA
Swiss Federal Institute of Technology Lausanne, Switzerland
The Netherland Cancer Institute, Amsterdam, The Netherlands
Triemli Hospital, Zurich, Switzerland
Universitätsklinikum Freiburg, Freiburg im Breisgau, Germany
University of Basel, Switzerland
University of Bayreuth, Germany
University of California, USA
University of Cambridge, UK
University of Cologne, Germany
University of Dundee, Scotland, UK
University of Edinburgh, UK
University of Geneva, Switzerland
University of Illinois, Chicago, IL, USA
University of Konstanz, Germany
University of Lausanne, Switzerland
University of Miami, USA
University of Michigan, USA
University of Milano, Italy
University of Minnesota, USA
University of Nottingham, UK
University of Oslo, Rikshospitalet, Norway
University of Oxford, England, UK
University of Padua, Italy
University of Pennsylvania School of Medicine, USA
University of Pittsburgh, USA
University of Sussex, UK
University of Texas Health Science Center, San Antonio, USA
University of Toronto, Canada
University of Trento, Italy
University of Ulm, Germany
Vanderbilt University, Tennessee, USA
VU University Amsterdam, The Netherlands
Washington University School of Medicine, USA
Wayne State University, Detroit, USA
Yale University, New Haven, USA

Awards

Alessandro Sartori was awarded the Ernst Th. Jucker Prize 2008.

Teaching

Jiricny Josef
teaches in the Mantelstudium "Biomedizinische Wissenschaften GDE I" for 2nd year medical students.

teaches parts of graduate students' block-course in basic cancer research for students of Cancer Biology PhD Course.

teaches parts of graduate students' block-course in basic cancer research for students of the Molecular Life Sciences PhD Course.

Marra Giancarlo
organizes laboratory training for PhD and diploma students at the Institute of Molecular Cancer Research and seminars at the Institute of Molecular Cancer Research.

Müller Anne
teaches the course „Molekulare Zellbiologie II" for 1st year medical students in the summer and winter semesters.

teaches parts of graduate students' block-course in clinical cancer research.

teaches a two-day course per semester on scientific writing.

Schwendener Reto
teaches POL Tutorial Molecular Cell Biology at the Medical Faculty of University of Zurich.

supports college students in performing their graduation thesis (Maturaarbeit). Swiss Academy of Science (Akademie der Naturwissenschaften Schweiz, SCNAT). 4-6 Students per year.

Janscak P., Lopes M., Marra G., Müller A., Sartori A. and Schwendener R.
teach course BIO246 "Genome Instability and Molecular Cancer Research" for Biology students at the University of Zurich. Lopes M. is coordinator of this course.

Conferences

2008

Engels Kim
Regulation of Exonuclease-1 by protein interactions
3rd Cancer Research Retreat, Fiesch, Switzerland

Ferrari Stefano
Regulation of hEXO1 by post-translational modifications
Dept. of Biomolecular Sciences and Biotechnology, University of Milano, Italy

The control of cell division by protein phosphorylation
Faculty of Science, University of Tanta, Egypt

The control of cell division by protein phosphorylation
Institute of Biochemistry, Faculty of Science University of Cairo, Egypt

The DNA damage response
Institute of Biochemistry, Faculty of Science, University of Cairo, Egypt

DNA damage and the control of cell cycle transitions
Institute of Biomedical Investigations "Alberto Sols", CSIC, University of Madrid, Spain

Post-translational modifications controlling hEXO1: a paradigm to investigate the DNA damage response
Novartis Pharmaceuticals, Department of Oncology, Basel, Switzerland

Janscak Pavel
Biochemistry and Function of RECQL5
Molecular and Clinical Mechanisms in Bloom's Syndrome and Related Disorders
University of Chicago's Gleacher Center, Chicago, USA

Jiricny Josef
Mismatch repair at the crossroads
D-Biol Symposium, Davos, Switzerland

DNA Mismatch Repair and DNA damage signalling
EU Progress, Rotterdam, The Netherlands

Roles of MMR and BER in the processing of O6-methylguanine and fluorouracil
Mexico

When DNA repair kills: cytotoxic processing of methylation damage and 5-fluorouracil
OOA-course Replication stress and genome maintenance, NKI-AVL Amsterdam, The Netherlands

DNA Mismatch Repair and its Defects in Familial and Sporadic Colon Cancer
Paris, France

The mismatch repair interactome
Prague, Czech Republic

BER and MMR in SHM
Russian-European Workshop on DNA Repair and Epigenetic Regulation of Genome Stability, St. Petersburg, Russia

Lopes Massimo
EM analysis of DNA replication intermediates
Analysis of DNA replication and genomic instability by DNA combing and other single-molecule assays, Saint-Raphael, France

Marra Giancarlo
Colon cancers with defects of the DNA mismatch repair
5th DNA repair Workshop, Smolenice Castle, Slovak Republic

DNA mismatch and colon cancer
Clinical Oncology, Biomodul 415 - University of Zurich, Switzerland

Dynamics and Maintenance of the Genome: DNA Replication, repair
Course H5, University of Basel, Switzerland

Clinical and molecular characterization of subjects at risk of colon cancer
II International Workshop on Colorectal Cancer, Regina Elena Institute, Rome, Italy

Colorectal Adenocarcinoma: Not one disease anymore
Neue Forschungsergebnisse in der Oralen Onkologie, University of Zurich, Switzerland

Impairement of the DNA mismatch repair in familial and sporadic colon cancer
PhD Course: Molecular Medicine in Cancer and Immunology, University of Oslo, Norway

Mutation of the colon cancer landscape by mismatch repair defects
Symposium "Kolonkarzinom: Neues aus Dianostik und Therapie", University of Zurich, Switzerland

Epithelial colorectal cancer: Not one disease anymore
URPP in System Biology / Functional Genomics, Ascona, Switzerland

Müller Anne
T-cell responses in *Helicobacter*-induced gastric preneoplastic pathology
8th International Workshop on Pathogenesis and Host response in *Helicobacter* infections, Lo-skolen, Helsingor, Denmark

T-cell responses in *Helicobacter*-induced gastric preneoplastic pathology
Minisymposium on infectious diseases, Max-Planck-Institute Infection Biology, Berlin, Germany

T-cell responses in Helicobacter-induced gastric preneoplastic pathology
URPP in System Biology / Functional Genomics, Ascona, Switzerland

Schwendener Reto
Vaccine formulations with liposomes and other drug delivery systems
Cytos AG, Schlieren, Switzerland

The tumor microenvironment as new target fro cancer therapies
Gynäkologische Forschung: Neue Ergebnisse - 1016, USZ, Switzerland

2009

Ferrari Stefano
The eukaryotic cell division cycle: regulatory aspects and the connection to cancer
Probevorlesung for the Habilitation at the Faculty of MNF, University of Zurich, Switzerland

Ghodgaonkar Medini
Are mutations alone sufficient to give rise to cancer?
4. Cancer Research Retreat, Ascona, Switzerland

Hitzler Iris
Towards a rational vaccination approach against *Helicobacter pylori*
4. Cancer Research Retreat, Ascona, Switzerland

Rational Development of a *Helicobacter Pylori* Vaccine
7th Helicobacter-Workshop, Deutsche Gesellschaft für Hygiene und Mikrobiologie, Goslar, Germany

Janscak Pavel
Role of RECQ5 DNA Helicase in Maintenance of Genomic Stability
EMBO conference: "Helicases and NTP-Driven Nucleic Acid Machines: Structure, Function and Roles in Human Disease" Les Diablerets, Switzerland

Jiricny Josef
Interference of BER and MMR in SHM
Mismatch-to-Model EU Meeting, Amsterdam, The Netherlands

The Mismatch Repair Interactome
DNA Damage Response and Repair Mechanisms, Crete

The mismatch repair interactome
Systems Level Understanding of DNA Damage Responses, Egmund-am-See, The Netherlands

Interference of base- and mismatch repair in somatic hypermutation
International Congress on Environmental Mutagenesis, Florence, Italy

Interference of base- and mismatch repair in somatic hypermutation
CIG Symposium 2009: DNA repair and Human Health Lausanne, Switzerland

Base- and mismatch repair interference in somatic hypermutation
Swiss-Japanese Workshop on DNA Metabolism, Villars, Switzerland

Kumar Sushil, Mete Sibel, Schwendener Reto
Aminobisphosphonate liposomes polarize tumor associated macrophages from M2 to M1 phenotype and reduce tumor growth in a mouse tumor model
23rd annual Meeting of the European Macrophage and Dendritic Cell Society (EMDS), Regensburg, Germany

Lopes Massimo
Structural Insights into Genome Instability associated to DNA replication stress
Switzerland-Japan join meeting: Chromosome Dynamics and Genome Stability, Switzerland

Marra Giancarlo
Molecular insights into colorectal tumor biology gained through transcriptomic studies
International Sabbatical staging, "Casa di Cura Sollievo della Sofferenza" of S. Giovanni Rotondo, Italy

The DNA changes that lead to inherited diseases and cancer International Sabbatical staging, "Casa di Cura Sollievo della Sofferenza" of S. Giovanni Rotondo, Italy

Impairment of the DNA mismatch repair in familial and sporadic colon cancer International Sabbatical staging, "Casa di Cura Sollievo della Sofferenza" of S. Giovanni Rotondo, Italy

The DNA changes that lead to inherited diseases and cancer
Lecture for the acquisition of the title of Privatdozent at the University of Zurich, Switzerland

Molecular insights into colorectal tumor biology gained through transcriptomic studies
Workshop on Gastrointestinal Oncology, Porto Ercole, Italy

Mete Sibel
Aminobisphosphonate liposomes polarize tumor associated macrophages from M2 to M1 phenotype and reduce tumor growth in a mouse tumor model
4. Cancer Research Retreat, Ascona, Switzerland

Mihaljevic Boris
Role of mismatch repair proteins in response to double-strand breaks in mammalian cells
4. Cancer Research Retreat, Ascona, Switzerland

Müller Anne
Neonatal infection with CagA$^+$ *H. pylori* induces tolerance and prevents gastric cancer
Annual Meeting of the German Society for Hygiene and Microbiology, Goettingen, Germany

T-cell responses in *Helicobacter*-induced gastric preneoplastic pathology
Department of Pathology, Univiversity of Zurich, Switzerland

The role of T-cells in the progression of *Helicobacter*-induced gastric cancer and gastric mucosa-associated lymphoid tissue lymphoma
Helicobacter workshop, German Society for Hygiene and Microbiology, Goslar, Germany

The role of T-cells in the pathogenesis of *Helicobacter*-induced gastric cancer and gastric mucosa-associated lymphoid tissue lymphoma
Institute of Immunobiology, St. Gallen, Switzerland

T-cell responses in *Helicobacter* -induced gastric neoplasia
World Immune Regulation Meeting, Davos, Switzerland

Oertli Mathias
Characterisation of the transgenic DC-SIGN mouse
4. Cancer Research Retreat, Ascona, Switzerland

Ray Chaudhuri Arnab
Uncovering the structural determinants of DNA replication stress induced by topoisomerase inhibition
4. Cancer Research Retreat, Ascona, Switzerland

Sayi Ayca
Dual role of IFN-gamma in Helicobacter-induced gastric preneoplastic lesions
Turkish National Immunology Congress, Turkey

Seminars

2008

Bonde Jesper
Virus Elisa Laboratory, Department of Virology, Statens Serum Institute, Copenhagen, Denmark
Clinical Utility of Microarray Based Human Papilloma Virus Diagnostics: Improved Cervical Cancer Prevention Programme Monitoring HPV Vaccine Impact

Chapman Ross
Wellcome Trust and Cancer Research UK Gurdon Institute, University of Cambridge, UK
Co-ordination of the chromatin response to DNA double-strand breaks by MDC1

Constantinou Angelos
Department of Biochemistry, University of Lausanne, Switzerland
Mechanisms of tumor suppression by the Fanconi anemia pathway

Dianov Grigory
MRC Radiation Oncology and Biology Unit, Harwell, Didcot, Oxfordshire, UK
Regulation of base excision repair in response to DNA damage

Dogliotti Eugenia
Unit of Molecular Epidemiology, Istituto Superiore di Sanita, Rome, Italy
Downregulation of base excision repair in terminally-differentiated cells: how cells deal with accumulation of DNA damage?

Giglia-Mari Giuseppina
Department of Cell Biology Genetics, ErasmusMC, Rotterdam, The Netherlands
TFIIH "revisited"

Hopfner Karl-Peter
Gene Center and Department of Chemistry and Biochemistry, University of Munich, Germany
DNA repair processes at atomic resolution

Kovalchuk Olga
Department of Biological Sciences, University of Lethbridge, Alberta, USA
Role of epigenetic deregulation in breast cancer chemoresistance

Kunkel Thomas A.
NIEHS, Research Triangle Park, NC, USA
Studies of leading and lagging strand DNA replication fidelity in yeast

Liskay Michael
Oregon Health Science University, Portland, Oregon, USA
A stochastic 'Cre-lox' system for studying normal and abnormal mouse development

Llorca Oscar
Centre for Biological Research, Spanish National Research Council, Madrid, Spain
3D structure and function of macromolecular multi-protein complexes involved in DNA repair using electron microscopy

Meyer Hemmo
Institute of Biochemistry, ETH-Zurich, Switzerland
The ubiquitin-dependent Cdc48/p97 chaperone system control mitosis and genome stability

Petrova Tatiana
Centre Pluridisciplinaire d'Oncologie, CHUV and University of Lausanne, Switzerland
New kid on the block: PROX1 in the control of colon cancer progression

Plevani Paolo
Dipartimento di Scienze Biomolecolari e Biotecnologie, University of Milan, Italy
Converging mechanisms for the full activation of the DNA damage checkpoint

Ramsay Rob
Peter MacCallum Cancer Centre, East-Melbourne, Australia
Pathways that regulate gastrointestinal homeostasis that are subverted in disease

Rass Ulrich
Genetic Recombination Laboratory, Cancer Research UK, South Mimms, UK
Aprataxin and links to human disease

Sartori Alessandro
Institute of Molecular Cancer Research, University of Zurich, Switzerland
Cell cycle regulation of DNA double-strand break repair by CtIP/SAE2

Stucki Manuel
Institute of Veterinary Biochemistry and Molecular Biology, UZH, Zurich, Switzerland
MDC1 and yH2AX: linking the DNA damage response machinery to broken chromosomes

Yepiskoposyan Hasmik
Genome Research Institute, University of Cincinnati, Ohio, USA
Metabolism and survival downstream of Akt/PKB

Young Graeme P.
Flinders Medical Centre, Bedford Park (Adelaide), Australia
The search for faecal markers of colorectal neoplasia

2009

Balestrini Alessia
Genome Stability Unit Clare Hall, Cancer Research UK, UK
GCC1, a novel protein required for initiation of chromosomal DNA replication

Beard Peter
EPFL, Lausanne, Switzerland
The DNA damage response to adeno-associated virus and its effect on tumour cells

Branzei Dana
DNA repair lab., F.I.R.C. Institute of Molecular Oncology Milan, Italy
Factors implicated in recombination-mediated damage bypass involving sister chromatid junctions and their regulation

Breivik Jarle
University of Oslo, Norway
Have the DNA mismatch repair phenotypes shaped their own genotypes?

Demple Bruce
Department of Genetic and Complex Diseases, Harvard University, Boston, USA
Avoiding Radical Entanglements: Repair of Oxidative DNA Damage in the Nucleus and Mitochondria

Durandy Anne
INSERM unit 429 at Necker University Hospital, Paris, France
Class switch recombination through the study of inherited immunodeficiencies

Fernandez-Capetillo Oscar
Spanish National Cancer Research Centre (CNIO), Madrid, Spain
Exploiting ATR knowledge for tumor elimination, and chromatin/MMR stories

Groth Anja
Biotech Research and Innovation Centre University of Copenhagen, Denmark
Histone Dynamics and DNA Replication

Hickson Ian
The Weatherall Inst. of Molecular Medicine, University of Oxford, UK
Genomic instability and cancer: lessons from analysis of Bloom's syndrome

Hirota Kouji
Department of Radiation Genetics, Graduate School of Medicine, Kyoto University, Japan
Transcription of mRNA-type long non-coding RNAs (mlonRNAs) disrupts chromatin array

Kisker Caroline
Rudolf Virchow Center for Experimental Biomedicine, University of Würzburg, Germany
New insights into nucleotide excision repair - Crystal structure of the FeS cluster containing DNA helicase XPD

Pedersen Susanne
Clinical Genomics, North Ryde, New South Wales, UK
Discovery and validation of biomarkers for colorectal neoplasia

Ried Thomas
Genetics Branch, Center for Cancer Research, NCI, NIH, DHHS, Bethesda, Maryland, USA
Causes and consequences of chromosomal aberrations in cancer cells

PhD Students
Isabelle Arnold
Payal Bhatia
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Vanessa Craig
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Kim Engels
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David Fischer
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Mario Gysi
Sabrina Heiny
Liyan Hu
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Valery V. Ilinsky
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Leah Pogorzala
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Ildem Sanli
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Janny Tilma
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* left IMCR during 2008/9