INSTITUTE OF MOLECULAR CANCER RESEARCH

SCIENTIFIC REPORT 2006/7

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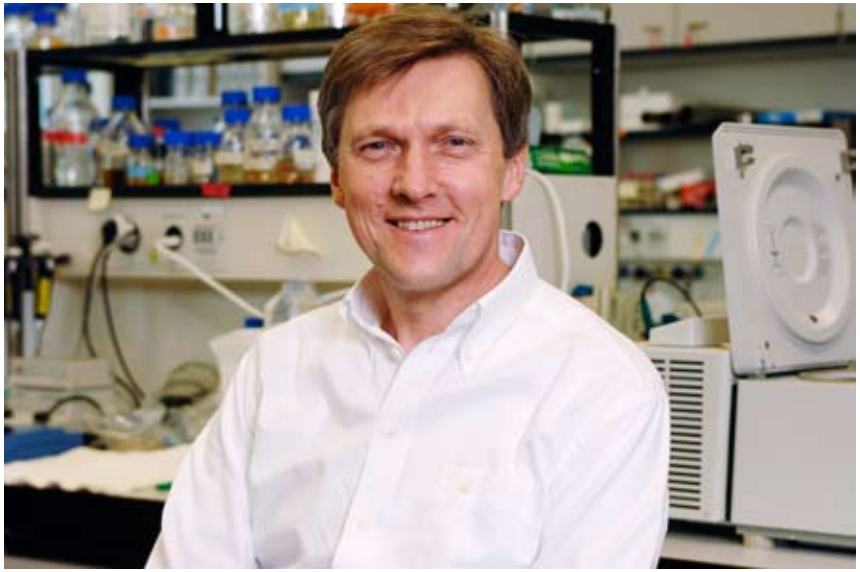
Foreword



Josef Jiricny

Director

The Institute of Molecular Cancer Research (IMCR) has enjoyed its first couple of years on the Irchel Campus of the University. This has been a very positive experience, with many possibilities for interaction with other institutes, specifically the Institute of Veterinary Biochemistry and Molecular Biology (IVBMB), which is housed on the floor above and the Institute of Molecular Biology (IMB), which is in the neighbouring building. The ready access to the numerous facilities and technology platforms of the University, such as the Functional Genomic Center Zurich (fgcz.uzh.ch) has also substantially facilitated our work.

We were able to welcome three new members of staff, Massimo Lopes, Anne Müller and Reto Schwendener. Massimo, a senior potdoctoral assistant in my laboratory, won the prestigious Assistant Professor position sponsored by the Swiss National Science Foundation. Anne is a tenure-track Assistant Professor for Functional Genomics of Cancer, who joined us from Stanford University and who is supported by the University Research Priority Program in Systems Biology/Functional Genomics. Reto came to us from the Paul Scherrer Institute.

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. In only a few intakes it has grown into the second 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 (cnz.uzh.ch) and other research groups of the University and the Swiss Federal Institutes of Technology (ETH).

With its new intake, the Institute has now grown to nearly 60 persons, 11 of whom (3 senior scientists, 2 administrative and 6 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. Unfortunately, this increase in personnel has been countered by a reduction in the Institute's budget, imposed across the board by the University, which means that the senior scientists have to spend more time writing grant applications instead of being actively involved in re-

search and teaching. But this is a fate common to many institutes these days.

Our long-term goal is to position ourselves among the leading institutes of molecular cancer research in the world. Given that the Institute still has room to accommodate one or two more groups, we are actively searching for promising young candidates interested in setting up their first groups in our midst. It is foreseen that these positions will be filled during 2008. We are also continuing our efforts to create a second Chair of Cancer Research and are actively seeking ways of financing this position.

Until last year, the principal focus of the Institute has been on the study of the mechanisms of DNA repair and DNA damage signalling. With the arrival of Anne Müller, we have extended our efforts in the field of gastrointestinal malignancy, which has been represented by the Marra group in the form of the hereditary non-polyposis colon cancer (HNPCC) projects, and which will substantially profit from the increased critical mass in gastrointestinal cancer brought in by Anne and her group. Reto Schwendener brings with him a great deal of experience in liposome technology and his translational research group represents an important link to the clinic.

Our efforts in transcriptomics and proteomics have helped us identify very interesting novel tumour markers and potential future therapeutic targets, which interact with DNA repair proteins, but which are likely involved in other cellular pathways. We intend to follow up a few of these novel leads, such that the emphasis on DNA repair may diminish somewhat in favour of these new fields of study.

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.

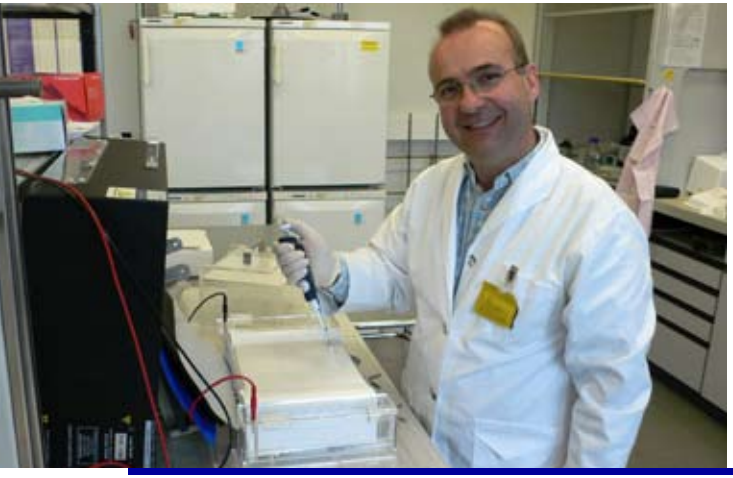
Scientific Advisory Board

IMCR is regularly reviewed by its Scientific Advisory Board, which consists of:

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

Recent studies have shown that a variety of stimuli deriving from the environment or originating from within the cell impinge on the machinery that drives the transition through the cell division cycle. Transduction of such signals within cells relies on rapid and reversible post-translational modification of proteins. The goal of our work is to elucidate pathways that are triggered in response to DNA damage and signal to the cell cycle machinery. In particular, two aspects are under scrutiny: (i) the identification and the role of post-translational modifications of proteins involved in DNA synthesis and DNA repair; (ii) the effect of DNA damage on the onset of mitosis.

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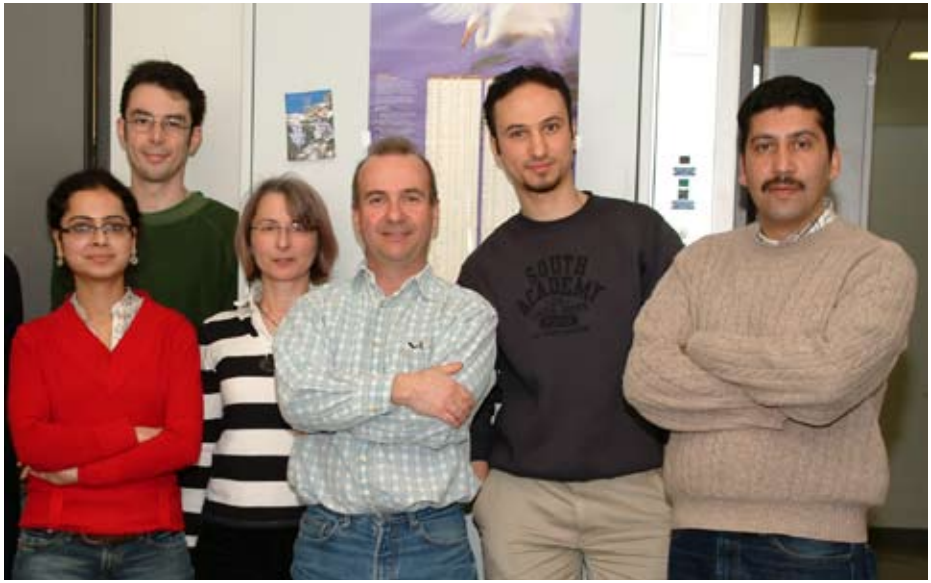
Ines Lohse

Technician
Christiane König



DNA damage can be caused by exogenous agents, such as inhaled cigarette smoke, ultraviolet light and dietary factors, or may result from endogenous metabolic processes. Failure to detect and correct DNA damage before cell division results in genetic instability observed in 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 "DNA damage response" or DDR.

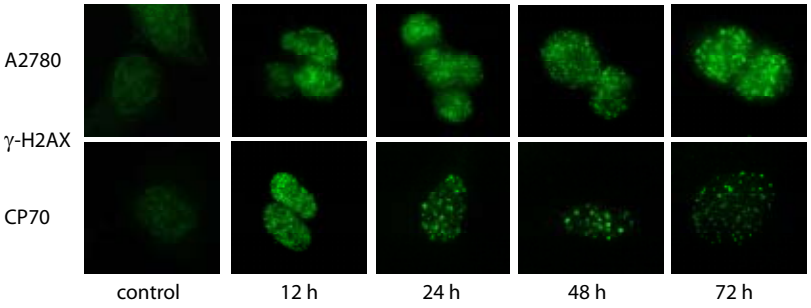
Curiously, despite DNA the fact that damaging agents pose a threat to the genome, they still represent the treatment of choice in cancer therapy. The rationale for the use of radiation or radiomimetic drugs in therapy was the argument that they eliminate cancer cells by triggering apoptosis. However, this dogma has been recently challenged by studing the behavior of cancers that develop in the human body. The essence of the challenging argument is that solid cancers are unlikely to retain the ability to self-destruct by apoptosis because it is precisely through inactivation of the apoptotic machinery and promotion of aggressive vascularization that cancer cells succeed to recover from latency and are able to form solid tumors. This suggests that radiation and drugs that cause tumor shrinkage *in vivo* must operate by mechanisms other than apoptosis. Indeed, oncologists and radiation biologists have observed that in tumors undergoing radio- or chemotherapy, cells die when they try to divide and, typically, daughter chromosomes break when they attempt to separate during mitosis. For this reason, significant effort in basic research and drug discovery programs is currently put on the elucidation of DDR pathways and the checkpoints they trigger. It is believed that in the near future, treatment of cancer patients with a combination of tolerable dosages of conventional radio- or chemotherapy and selective drugs that inactivate key cell cycle checkpoints will likely be the avenue to achieve clearance, or at least significant shrinkage, of solid tumors.

Mismatch repair status and the response of human cells to cisplatin

Elisabetta Pani, Mahmoud El-Shemerly, Christiane König

The emergence of resistance to cisplatin is a serious drawback of cancer therapy. To help elucidate the molecular basis of this resistance, we examined matched cancer cell lines that differ in their response to cisplatin. Checkpoint activation by cisplatin appeared to be identical in sensitive- and resistant-cells, independently of their DNA mismatch repair (MMR) status. However, cell cycle analysis revealed that cisplatin-sensitive (and MMR-proficient) cells delayed S-phase transition, arrested at G2/M and died by apoptosis, whereas resistant (and MMR-deficient) cells transiently arrested at G2/M but ultimately resumed cell cycle progression. Only in cisplatin-sensitive cells homologous recombination (HR) proteins were selectively degraded. Our data indicate that although MMR undoubtedly contributes towards the cytotoxicity of cisplatin, it is only one of several pathways that modulate the cellular response to this drug. On the other hand, HR appears to be important in cisplatin cytotoxicity and the HR status might represent a novel prognostic marker and possibly also a therapeutic target, the inhibition of which would substantially sensitize cells to cisplatin chemotherapy.

Figure 1.
Response to cisplatinum in the ovarian cancer cell line A2780 (sensitive) and the subline CP70 (resistant) as evidenced by the formation of γ-H2AX foci.



Regulation of hEXO1 in response to replication fork stalling

Mahmoud El-Shemerly, Aswin K. Pyakurel, Said Mosehly

Nucleases play important roles in DNA transactions. Human Exonuclease 1 (hEXO1) participates in mismatch repair, homologous recombination and DNA replication. We have previously shown that, in response to stalled replication, hEXO1 is phosphorylated and, as a consequence, undergoes ubiquitination and degradation. Using chemical inhibitors, RNA interference and ATM- and ATR-deficient cell lines, we have obtained evidence that hEXO1 phosphorylation is ATR-dependent. By means of liquid chromatography-tandem mass spectrometry (LC-MSMS) we have identified 9 sites of phosphorylation in hEXO1 and 3 additional sites upon treatment with hydoxyurea (HU). We have raised an antibody to pS_{714}, an S/T-Q type-site, that is commonly modified by ATM and ATR, and we obtained evidence that S714 is phosphorylated in response to HU- but not IR-treatment.

Our data, together with evidence published in the literature, suggest a scenario according to which the uncoupling of DNA synthesis and DNA unwinding at stalled forks generates structures that are suitable for processing by hEXO1. Such intermediates are capable of triggering ATR/CHK1-dependent signals. ATR-dependent phosphorylation of hEXO1, in turn, provides the means to direct EXO1 to degradation. This would result in the stabilization of stalled forks (Figure 2).

Ongoing work in the laboratory aims at uncovering the ubiquitin-dependent pathway responsible for hEXO1 degradation at sites of stalled replication.

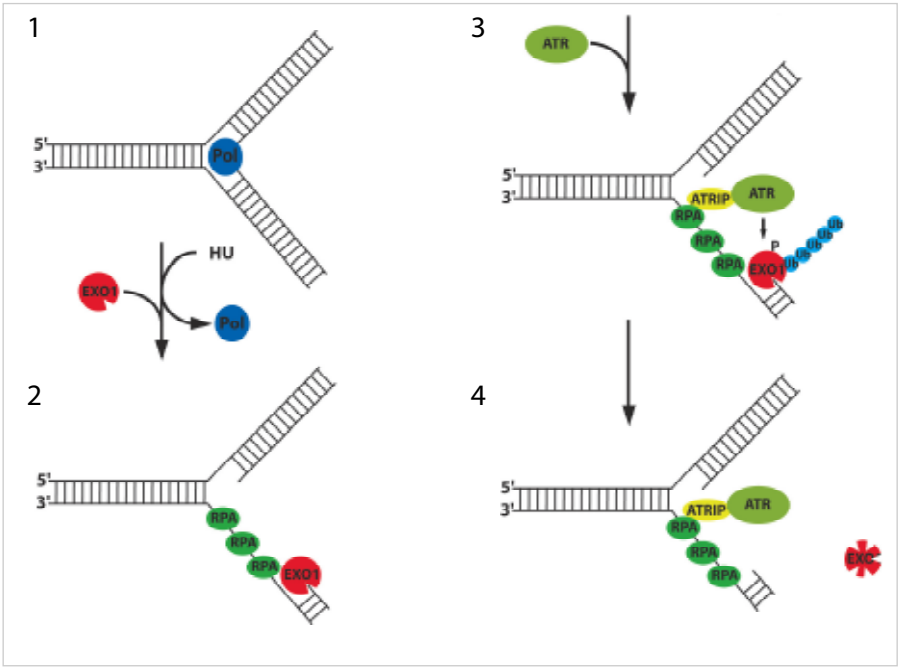


Figure 2.
Schematic model of the control of hEXO1 at stalled replication forks.

Control of hEXO1 by protein-protein interactions: 1. Mass Spectrometry

Mahmoud El-Shemerly, Aswin K. Pyakurel, Wassim Eid

To specifically identify proteins binding hEXO1 and possibly involved in its poly-ubiquitination, we have performed liquid chromatography-mass spectrometric (LC-MS) analysis of hEXO1-interacting proteins. In order to immunoprecipitate hEXO1 from cells treated in the presence or absence of HU we have employed either an affinity-purified antibody to the full-length protein or an antibody to pS_{714} (see previous page). This approach has provided a number of potentially interesting partners, among which are replicative-helicases, DNA repair proteins and mediators of the DDR. Identification of previously described hEXO1 partners, such as DNA mismatch repair proteins, confirmed the reliability of this analysis. Of particular interest, this approach allowed identifying a RING-finger containing protein that may be either the E3-ligase directly responsible for EXO1 poly-ubiquitinylation or part of an E3-complex in charge of this function.

Control of hEXO1 by protein-protein interactions: 2. Two-Hybrid-Screen

Kim Engels, Mahmoud El-Shemerly

In addition to the MS as means of identifing proteins interacting with hEXO1, we have used a complementary approach, namely a yeast 2-hybrid screen. The yeast two-hybrid system was originally developed by Fields and Song (1989) as a genetic assay to detect protein-protein interactions in a cellular setting. To the end of identifying hEXO1 partners, we cloned full-length EXO1 (fl-hEXO1) and the C-terminal region of the protein (ΔN-hEXO1) into a pLexA bait-vector, creating a fusion of a DNA binding domain of the LexA transcription factor (DBD) and hEXO1. The ΔN-hEXO1 bait construct was used to screen a human blood peripheral cDNA library. The cDNA library was cloned into the pACT prey vector in order to create a fusion of an activation domain of the GAL4 transcription factor (AD) and the cDNA's encoding an entire collection of potential interacting proteins. Activation of the reporter gene was measured as growth on a selective medium and in a colorimetric assay. Sequencing of the positive clones identified chromatin assembly factors and proteins binding to Holliday junctions, among others.

DNA damage response and the onset of mitosis: role of Aurora-A

Payal Bhatia, Christiane König

Transition through mitosis is an obligate step in the cell cycle of normal and transformed cells. Mitotic kinases are the ultimate targets 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 recently demonstrated that AurA is target of DNA damage response signals. We have found that, in response to DNA damage, AurA activity is inhibited in a CHK1-dependent manner and that this likely occurs through phosphorylation at a single site in AurA. Inhibition of AurA, in turn, blocks the CDC25-dependent activation of CycB/CDK1 during mitosis (Fig. 3). Current work aims at elucidating the CHK1-dependent mechanism of AurA inhibition as well as at identifying AurA substrates through a proteomic approach.

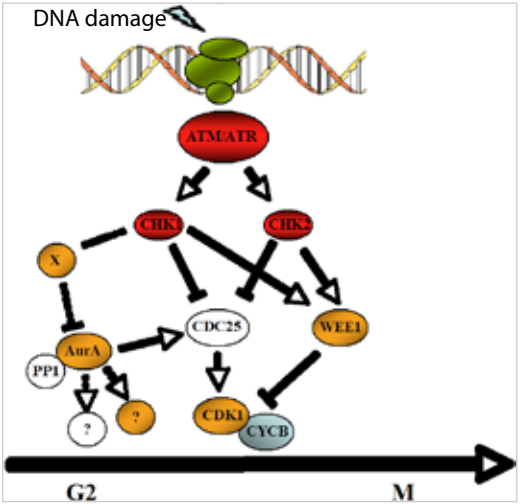


Figure 3.
Schematic representation of the pathways controlling Aurora-A.

These projects were supported by the Swiss National Science Foundation, the Désirée und Niels Yde Stiftung, Krebsliga Zürich, the Lydia-Hochstrasser-Stiftung, the Ida-de Pottère-Stiftung and the Sassella-Stiftung.

Pavel Janscak

Anti-recombination mechanisms

Homologous recombination (HR) provides a mechanism for accurate repair of DNA double-strand breaks (DSBs) and single-strand gaps during S/G2 phases of the cell cycle when the undamaged sister chromatid can serve as a template for repair. However, if executed improperly, HR can result in chromosomal rearrangements, a hallmark of tumorigenesis and tumor progression. The aim of our research is to advance our understanding of the molecular mechanisms involved in the suppression of aberrant recombination events.

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RecQ DNA helicases and maintenance of genomic stability

Kanagaraj Radhakrishnan, Lu Zheng, Sybille Schwendener, Daniela Hühn

RecQ DNA helicases are highly conserved in evolution and play important roles in the biological processes that enforce genomic stability. In humans, five RecQ homologues have been identified: RECQ1, BLM, WRN, RECQ4 and RECQ5 (Figure 1). Inherited mutations in the genes encoding for BLM, WRN and RECQ4 have been found to cause cancer-prone disorders with distinct clinical and cellular manifestations, suggesting that the multiple RecQ homologues in human cells have different biological roles.

Our major goal is to elucidate the cellular role of the human RECQ5 helicase. Recent genetic and cellular studies using mouse models have revealed that RECQ5 is a tumour suppressor that acts by preventing inappropriate HR events. In accordance with these findings, we have shown in collaboration with the laboratory of Dr. Patrick Sung that RECQ5 physically interacts with the RAD51 recombinase and has the ability to displace RAD51 from single-stranded DNA in a reaction dependent on ATP hydrolysis. These findings suggest that RECQ5 prevents HR via disruption of RAD51 presynaptic filaments. To prove this hypothesis, studies have been initiated to examine whether the interaction between RECQ5 and RAD51 is required for the suppression of HR *in vivo*.

We have also employed a proteomic approach to identify proteins that associate with RECQ5 *in vivo*. Specifically, we immunoprecipitated RECQ5 from extracts of human embryonic kidney cells using an affinity-purified rabbit polyclonal anti-RECQ5 antibody and analyzed the identity of co-precipitated proteins by mass spectrometry following their separation on SDS polyacrylamide gels. We found that RECQ5 was bound to the MRE11/RAD50/NBS1 (MRN) complex, which functions in many aspects of DNA metabolism involving DSBs. The cellular concentration of the RECQ5/MRN complex was not enhanced by DNA damage or by synchronizing cells in a particular stage of the cell cycle, suggesting a constitutive association. Experiments with purified proteins suggested that the association of RECQ5 with the MRN complex was mediated by a direct binding of RECQ5 to MRE11 and NBS1. Functional assays revealed that RECQ5 strongly inhibited the 3'-5' exonuclease activity residing in the MRE11 protein, suggesting that RECQ5 interacts with the nuclease domain of MRE11 and thereby prevents its interaction with the DNA substrate. At the cellular level, MRE11 was required for the accumulation of RECQ5 at sites of arrested replication forks and sites of laser-induced DSBs (Figure 2). Based on these findings, we hypothesized that the MRN complex recruits RECQ5 to sites of DNA damage and that these proteins work together to maintain genomic stability. Ongoing studies are addressing the possible role for the RECQ5/MRN complex in the repair of collapsed replication forks.

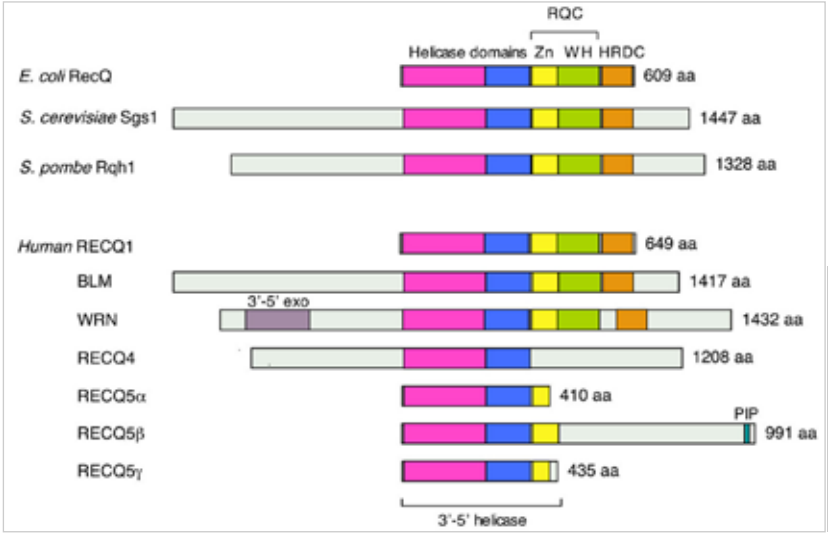

Figure 1.
RecQ family of DNA helicases. Schemes of selected members of the RecQ family are aligned relative to conserved domains. Zn, zinc-binding domain; WH, winged-helix domain; HRDC, Helicase and RNaseH C-terminal domain. RECQ5 exists in three different isoforms (α,β,γ) resulting from alternative RNA splicing. Only RECQ5β localizes to the nucleus and functions as a DNA helicase *in vitro*.

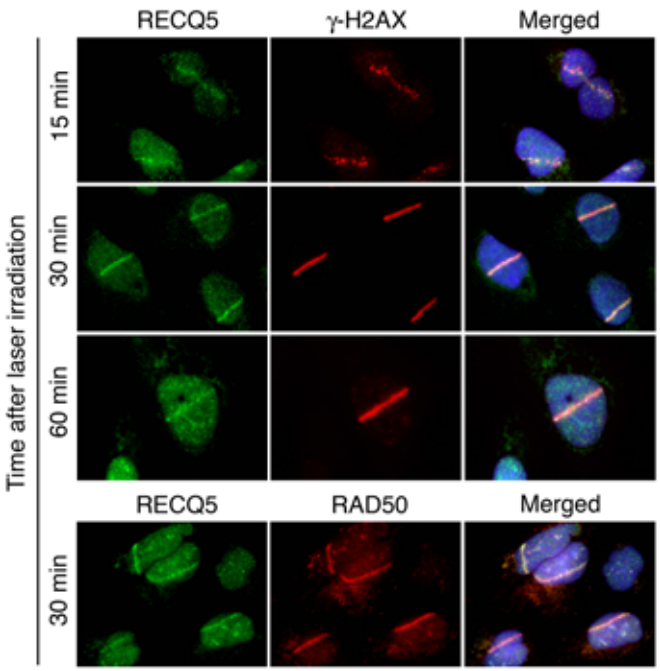

Figure 2.
RECQ5 accumulates at laser-induced DSBs. U2OS cells were sensitized with BrdU (10 μM; incubation for 24 hours) and subjected to microirradiation with a pulsed UV-A laser to generate linear tracks of DSBs. At the indicated time points after irradiation, the cells were fixed with methanol and co-immunostained either with anti-RECQ5 (green) and anti-γ-H2AX (red) antibodies or with anti-RECQ5 (green) and anti-RAD50 (red) antibodies. DAPI (blue) was used to stain the nuclei. γ-H2AX is a marker of DSBs.

Mismatch-repair system and DNA recombination

Javier Peña-Diaz, Boris Mihaljevic

It is well known that proteins involved in the initiation of post-replicative mismatch repair (MMR), such as MSH2, MSH6, MLH1 and PMS2, act during DSB repair to suppress recombination between homeologous (divergent) sequences. Moreover, there is evidence suggesting that the MMR proteins serve as components in the signaling events that activate cell-cycle checkpoints and apoptosis in response to DNA damage. Our aim is to help elucidate the molecular mechanisms underlying these processes.

We found that the MSH2/MSH6 heterodimer, termed MutSα, was avidly bound to the WRN helicase. This interaction was mediated by binding of MSH2 to the helicase domain of WRN. Functional studies indicated that MutSα strongly stimulated the helicase activity of WRN specifically on forked DNA structures with a 3′-single-stranded arm resembling strand-pairing intermediates of the single-strand annealing pathway of HR. The stimulatory effect of MutSα on WRN-mediated unwinding was enhanced by a G/T mismatch in the DNA duplex ahead of the fork, suggesting a mechanism in which MMR proteins work in conjunction with a DNA helicase to disrupt HR intermediates containing mismatches (Figure 3).

More recent studies in our laboratory revealed that, like WRN, the MMR proteins MSH2, MSH3, MSH6 and MLH1 were rapidly recruited to sites of laser-induced DSBs, arguing that these proteins associate with broken DNA ends prior to repair. By immunoFISH, we also observed that the MMR proteins MSH2 and MLH1 accumulated at telomeres specifically in cells that maintain telomeres by a recombination-based mechanism called alternative lengthening of telomeres (ALT) (Figure 4). Moreover, telomeric DNA was found to co-immunoprecipitate with MSH2 and MLH1 from extracts of ALT-positive cells, but not from extracts of ALT-negative cells. These findings suggest that the MMR proteins act at telomeres to regulate telomeric recombination. Our current studies focus on identifying the factors that are involved in the recruitment of MMR proteins to DSBs.

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

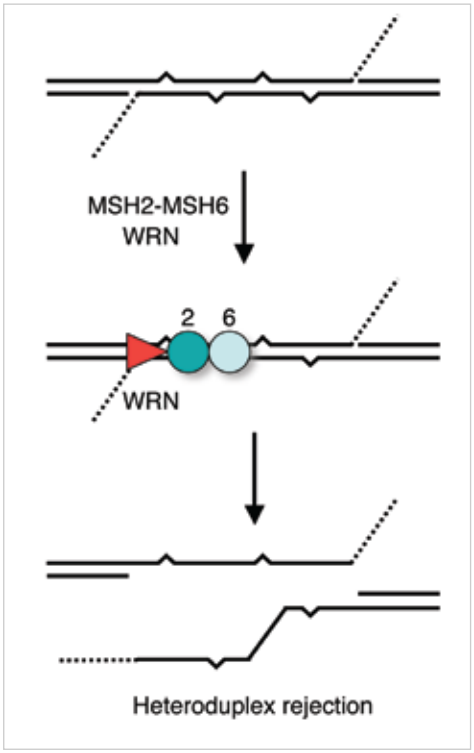

Figure 3.
Model for heteroduplex rejection during the single-strand annealing pathway of HR. The WRN helicase (red triangle) acts in conjunction with the MSH2/MSH6 mismatch recognition complex (green and blue spheres) to unwind recombination intermediates containing mismatches.

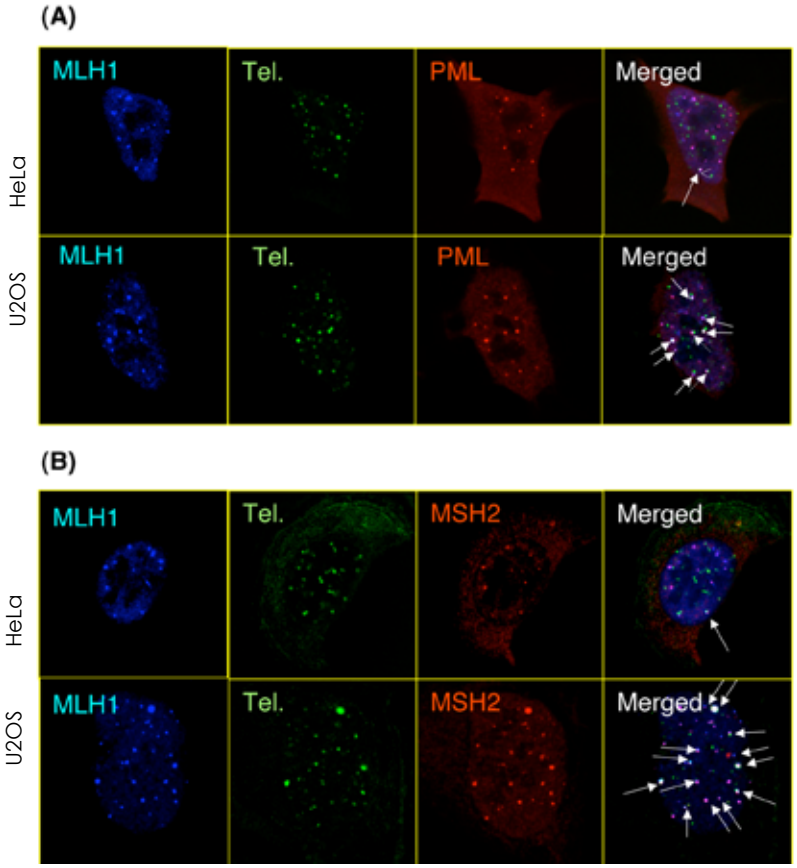

Figure 4.
MMR proteins accumulate at telomeres in ALT cells. (A) Immuno-FISH analysis of spatial distrubution of MLH1 (blue), PML (red) and telomeres (green) in U2OS cells (ALT+) and in HeLa cells (telomerase+) using anti-MLH1 and anti-PML antibodies and a FITC-labeled PNA probe for telomeric repeats. (B) ImmunoFISH analysis of spatial distribution of MLH1 (blue), MSH2 (red) and telomeres (green) in HeLa and U2OS cells. Images were captured by a confocal microscope.

Josef Jiricny

The multifaceted mismatch repair

During the past decade, 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, implicates MMR proteins also in other pathways of DNA metabolism and we are beginning to focus on some of these.

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Undergrad. Student
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Biochemistry of the human MMR system

Elda Cannavó, Bertran Gerrits (FGCZ), Patrick Kehl

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 in our laboratory. One of the aspects we plan to address 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 now plan to use these in the *in vitro* repair assays and test 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). The minimal reconstituted MMR system does not display quite the same substrate specificity as that observed in cell-free systems, which suggests that these modifications might play a role in repair. Alternatively, it is possible that some non-essential components of the MMR pathways are missing.

In an attempt to address the latter question, we carried out tandem affinity purifications (TAP-tagging) with extracts of stably-transfected cell lines expressing TAP-hMLH1 or TAP-hPMS2 in a background that lacks both wild type proteins. Expression of the TAP-tagged proteins complemented the MMR defect in these cells, which showed that the proteins are functional even with such large amino-, respectively carboxy-terminal extensions. The extracts of these cells were then passed through a series of affinity columns and the proteins associated with the tagged polypeptides were identified by mass spectrometric analysis of tryptic peptides. In the first series of experiments, we were able to identify all the known partners of hMLH1 and hPMS2. Making use of the more-sensitive new generation of mass spectrometers available at the Functional Genomics Center Zurich (www.fgcz.uzh.ch), we have now identified also a series of other possible partners of these polypeptides, some of which apparently do not participate in MMR. Some of these, e.g. RuvBL1, are under study in the laboratory (see below).

Repair of modified nucleotides

Petr Cejka, Franziska Fischer, Massimo Lopes, Nina Mojas

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 6-*O*-methylguanine (^{Me}G), such that MMR-deficient cells are up to 100-fold more resistant to killing by methylating agents of the S_N1 type than their MMR-proficient counterparts. MMR-deficient cells have also been reported to be resistant to cisplatin and many other chemotherapeutics, including 5-fluorouracil (FU). 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 a "nickase" – an enzyme capable of incising specifically only a single DNA strand of its recognition sequence. We succeeded in incorporating ^{Me}G and FU into our substrates, and were able to show that both substrates are addressed by the MMR system.

The FU/G mismatch was recognised and repaired as efficiently as T/G. Moreover, our results have shown that the FU/G mispair is addressed with high efficiency also by the base excision repair (BER) system. This suggested to us that the efficient metabolism of FU in DNA may contribute to the cytotoxicity of this important chemotherapeutic in situations where cells treated with the drug for a long time have depleted nucleotide pools and thus cannot fill-in the numerous repair patches generated by continuous excision of FU by the MMR and BER systems.

In contrast to FU/G, recognition of ^{Me}G mispairs with cytosine or thymine was found to vary greatly depending on sequence context and it was difficult to draw clear conclusions from our *in vitro* repair studies. However, with the help of electron microscopic evidence, we were able to show that genomic DNA of yeast and human cells treated with the prototypic methylating agent MNNG contained MMR-dependent single-stranded gaps at substantial

distances away from the replication forks. These findings substantiated our model, which posits that attempted processing of ^{Me}G by the MMR system leaves gaps in newly-replicated DNA due to the inability of the repair polymerase to find a perfect base-pairing partner for ^{Me}G. In the subsequent replication round, these gaps will cause collapse of the replication forks and trigger a cell cycle checkpoint and cell death.

The above findings suggested that processing of methylated bases leads to the generation of strand breaks. As these would be expected to be highly recombinogenic, we decided to see whether DNA recombination also contributes towards the cytotoxicity of methylating agents. To this end, we tested the sensitivity of recombination-deficient CHO cells to MNNG. Interestingly, these cells were found to be hypersensitive to killing by MNNG, and arrested already in the first S/G2 phase post treatment. Thus, recombination apparently helped rescue the cells after the first replication (Figure 1).

The involvement of DNA recombination in the processing of methylation damage led us to revisit the issue of susceptibility of *S. cerevisiae* to methylating agents. We could show that a MMR defect rescued the MNNG-sensitive phenotype of recombination-deficient yeast cells. This exciting finding enabled us to screen a library of 4'644 viable yeast deletion mutants (made available to us by Matthias Peter) for genes participating in MMR- and recombination-mediated processing of methylation damage. Some of the identified loci are subject of further investigation.

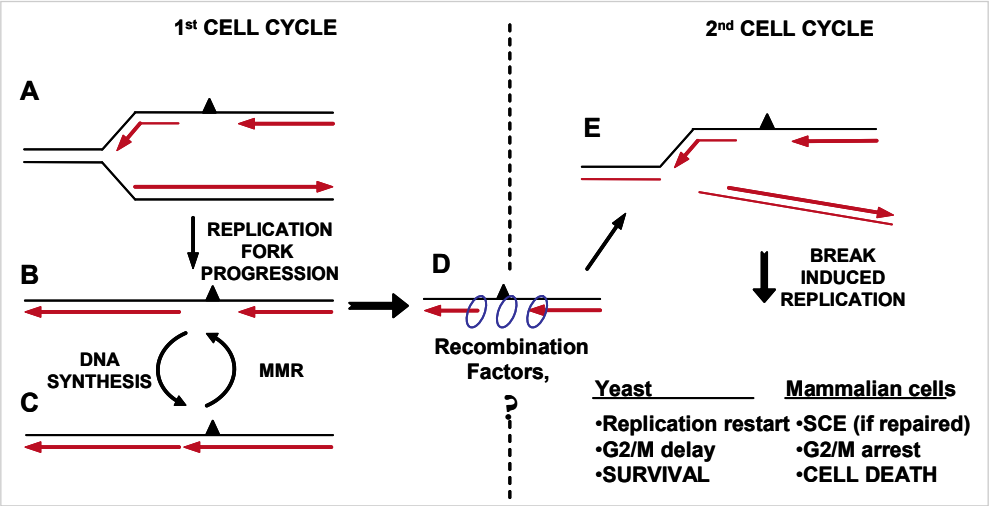


Figure 1.
Model for the roles of replication, MMR and recombination in DNA transactions induced by ^{Me}G.
When replication forks encounter ^{Me}G in the template DNA of MMR-proficient cells, they insert C or T and proceed with replication (A). The ^{Me}G-containing mispair activates MMR, which degrades the newly-synthesised strand up to and some distance past the ^{Me}G residue. This gap cannot be filled-in, because MMR repeatedly inhibits postreplicative gap repair (B, C). In the presence of functional recombination, the gaps are protected and progress to the next cell cycle (D), where they cause the collapse of replication forks (E). The collapsed replication forks can be restored with the help of HR, which leads to cell survival, at a cost of higher SCE levels. Cells that fail to rescue the forks arrest in G2/M and subsequently die due to their inability to restart replication.

Gene therapy of tumours with MMR defects

Kai Neelsen

The goal of this project was to test the feasibility of eliminating MMR-deficient tumour cells with the help of gene therapy. Because MMR defects give rise to microsatellite instability (MSI), open reading frames (ORFs) containing microsatellites will have a substantially greater propensity to acquire frameshift mutations than MMR-proficient cells. We therefore designed a "suicide gene" therapy approach, which consists of an enzyme/prodrug combination, in which the ORF encoding the enzyme is shifted out-of-frame by a microsatellite at its N-terminus. In a MMR-deficient cell, the vector carrying this expression cassette should be mutated with high frequency to give rise to molecules where the enzyme ORF was shifted into a correct reading frame. Treatment of these cells with an inactive prodrug will lead to their death, because they express the enzyme that converts the prodrug into a toxic metabolite. Normal cells will not die, in spite of having taken up the vector, because the open reading frame of the enzyme will remain out-of-frame thanks to the presence of the microsatellite repeat (Figure 2). Preliminary experiments deploying the thymidine kinase/ganciclovir combination in stably-transfected cells yielded very promising results and we are currently attempting to translate these findings into a virus-based gene therapy setting.

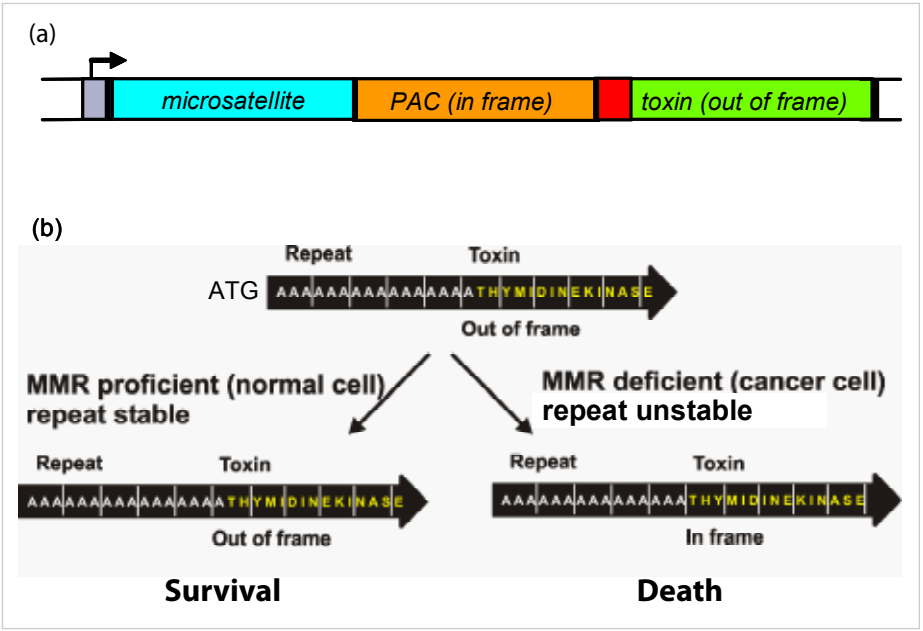


Figure 2.
Gene therapy approach based on in-frame shifting of a toxin gene. Provided that the results of the experiments described above are satisfactory, we will modify the PACeGFP assay system for possible therapeutic application.
(a) In the PAC-toxin fusion construct, the eGFP gene is replaced by a toxin gene, e.g. thymidine kinase.
(b) Scheme illustrating the rationale of our gene therapy approach. Frameshifts in the microsatellite of the PAC-toxin construct will restore the ORF of the toxin, leading to expression and subsequent elimination of MMR-deficient cells. Expression of the toxin will be monitored by a soft agar assay and should be detectable as a decrease in colony size and number due to the by-stander effect. The same assay will be used for testing of different toxins. Based on the results of the soft agar assay, we plan in vivo experiments using xenografts in nude mice.

MMR proteins in other pathways of DNA metabolism

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. We are attempting to obtain mechanistic insights into this important process of programmed mutagenesis by setting-up an *in vitro* system capable of mimicking the events *in vivo.*

Visualisation of MMR *in vivo* and *in vitro*

Rea Andermatt, Nina Mojas, Kai Neelsen

The eukaryotic MMR process has not been studied *in vivo*, largely due to the fact that mismatches in DNA cannot be induced like other types of DNA damage. However, now that we know that there are certain types of lesions (e.g. 5-bromouracil, 6-*O*-methylguanine) that are processed by the MMR system, we might be able to follow the assembly of the MMR repairosome *in vivo*. We intend to use direct and indirect immunofluorescence to visualise the different MMR proteins and their cofactors in cells following a variety of treatments. We first searched for foci in which the MMR proteins co-localise with other proteins responsible for DNA metabolism and repair. Particular attention was paid to polypeptides involved in DNA replication, recombination and lesion bypass. We also isolated stable clones expressing eGFP-hMLH1, eGFP-MSH2 and eGFP-MSH6. In collaboration with Jiri Lukas, we plan to study the assembly of the mismatch repairosome in living cells.

The role of the RuvBL1 helicase in genomic instability

Dennis Castor

We identified RuvBL1 as an interaction partner of TAP-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 may require its interaction with the MMR system. Surprisingly, indirect immunofluorescence studies showed that whereas 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 (Figure 3).

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

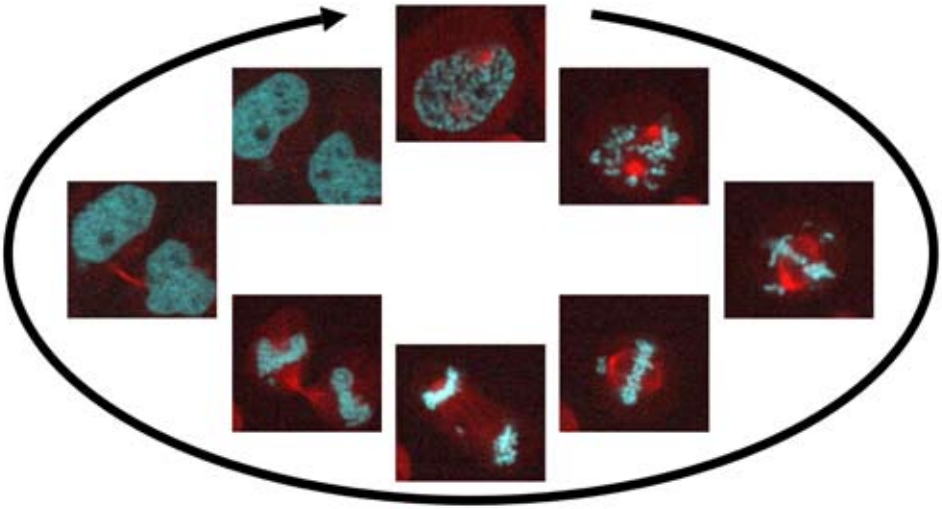


Figure 3.
Time-lapse confocal live-cell images of cells stably-expressing mRFP-tagged histone H2B (shown in cyan) and GFP-tagged α-tubulin (shown in red), in which RuvBL1 was knocked down by siRNA. This sequence of images shows a chromosome mis-aligned during metaphase and anaphase. Chromosomal instability and mis-segregation are key characteristics of cancer cells.

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, which may lead to cancer, as well as different neurological human syndromes.

Postdocs
Kai Neelsen

PhD students
Cindy Follonier
Arnab Ray Chaudhuri

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 DNA replication, relying on the generic observation that cancer cells, which frequently carry mutation in DNA repair and cell cycle checkpoint genes, are sensitized to DNA damaging agents. Despite the importance of replication stress in both cancer onset and therapy, little structural and genetic information about its underlying mechanisms in normal and cancer cells is available.

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 challenged by different types stress of (nucleotide depletion, damaged DNA) and to elucidate the role of checkpoint and DNA repair factors in these processes (Figures 1.-3.). During my last post-doctoral experience in the lab of Josef Jiricny at the IMCR, I could implement these techniques in the mammalian cell culture system, studying the cytotoxicity of a clinically relevant class of cancer chemotherapeutics (S_N1-type methylating agents).

I recently established an independent research group as a SNF Assistant Professor and chose the IMCR as the host institution. We are currently in the process of expanding the portfolio of available techniques to investigate DNA replication, in order to approach new biological questions related to genome instability, working in parallel in yeast and higher eukaryotic systems.

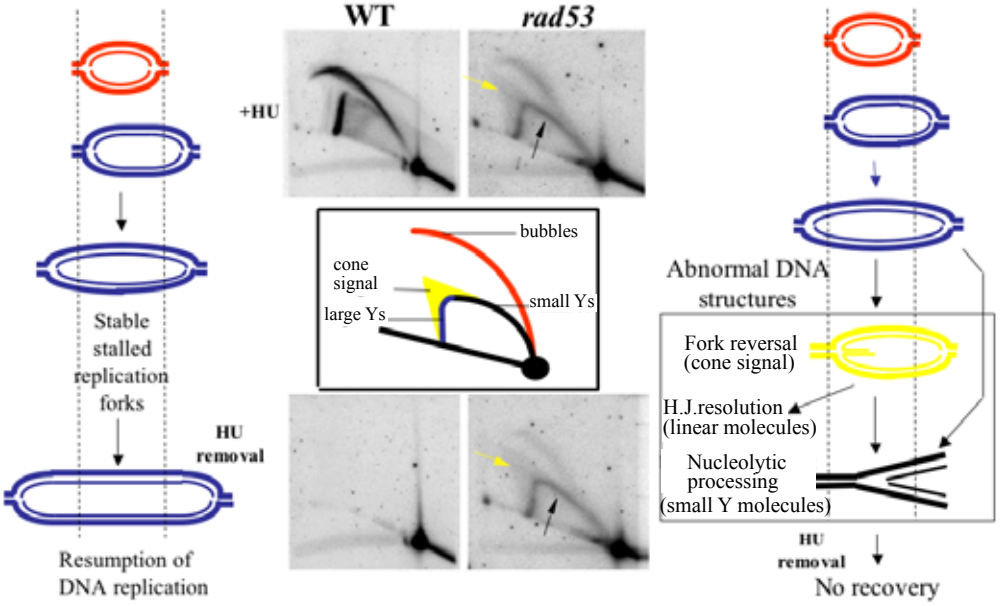


Figure 1.
Bi-dimensional electrophoretic analysis of replication intermediates (RIs) allowed to elucidate the crucial role of yeast checkpoint factors in stabilizing DNA replication forks stalled by hydroxyurea (HU)-induced nucleotide depletion. In HU-treated wt cells, standard RIs (bubbles and large Ys) accumulate on an early origin of replication (ARS305) and restart synthesis after HU-removal. In rad53 cells, standard RIs are barely detectable, while unexpected signals (complete Y ark and diffuse "cone signal") emerge at the origin and persist even after HU removal. The possible structure of the abnormal replication intermediates is depicted in the rectangle and is deduced by the position of the signals in the 2D gels. (Lopes et al., 2001. Nature 412:557)

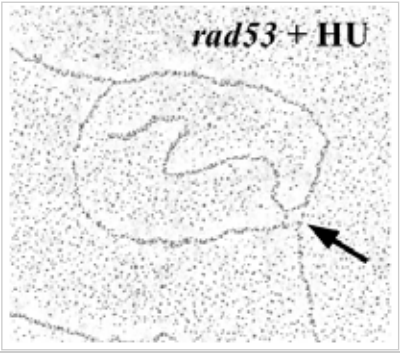
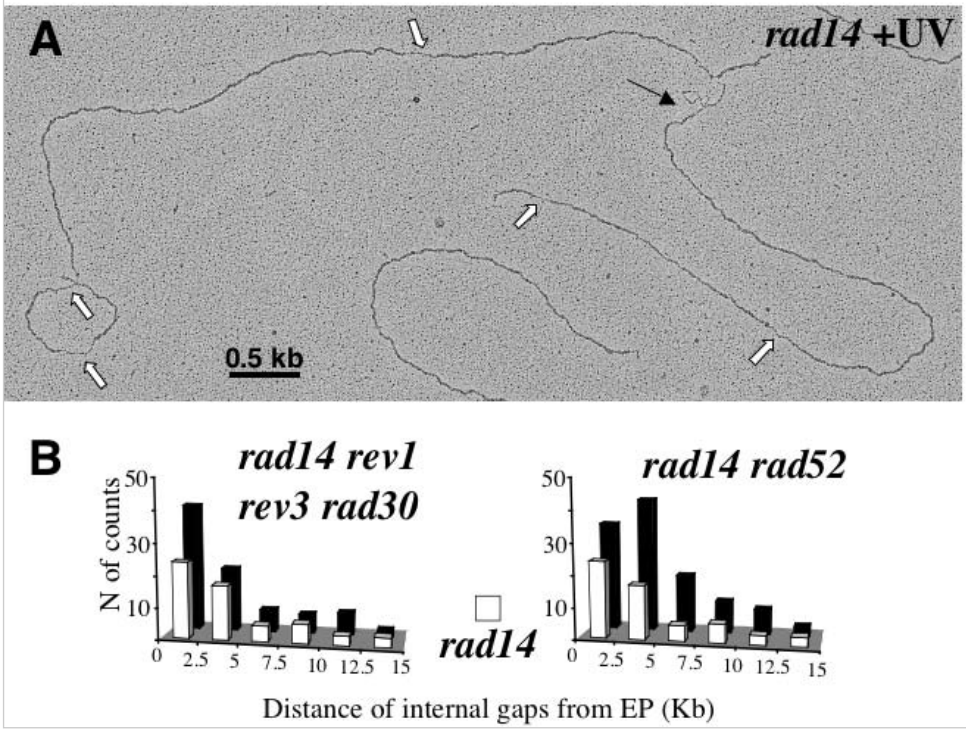


Figure 2.
Psoralen crosslinking coupled to Electron Microscopy (EM) provides further *in vivo* insights into the structure of replication intermediates. This EM micrograph shows a representative example of reversed fork ("chicken-foot" structure), detectable in about 10% of total stalled forks in HU-treated checkpoint-defective rad53 yeast cells. (Sogo et al., 2002. Science 297:599)

Figure 3. (below)
Extensive EM analysis of UV-irradiated nucleotide excision repair (NER)-defective (*rad14*) yeast cells allowed the visualization of long ssDNA region at the fork (A, black arrow), and smaller ssDNA gaps accumulating along the replicated duplexes at both sides of the fork (A, white arrows). The relative frequency and position of ssDNA gaps with respect to the elongation point (EP) can be compared in rad14 cells and in the multiple mutants where either translesion synthesis (rev1rev3 rad30) or homologous recombination (rad52) have been inactivated (B). These and other results led to a revised model of discontinuous DNA synthesis across a damaged template (C), challenging the dogma of semi-discontinuous DNA replication: whereas lagging strand synthesis can be readily restored after UV-lesion induced elongation blocks, leading strands transiently accumulate large ssDNA regions prior to replication restart. Proper repair of the resulting ssDNA gaps along replicated duplexes requires both translesion synthesis and recombination mechanisms. (Lopes et al., 2006. Mol Cell 21:15)



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 one of the earliest events in malignant transformation. In 2005, a number of 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 could 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 the products of these genes 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, how does altered DNA replication contribute to oncogene genotoxicity? 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? How do cells resolve these structures and escape the proliferative arrest?
This project aims to answer these open questions by providing structural information about replication intermediates isolated from the cell culture models of replication stress described above. We will employ a combination of cell, molecular and structural biology approaches (flow cytometry, radioresistant DNA synthesis, DNA fibre labelling, electron microscopy in combination with *in vivo*-psoralen crosslinking) to elucidate the nature of replicative stress and identify aberrant DNA structures that are responsible for DNA damage checkpoint activation.
At a later stage, we plan to extend our study to a mouse model of *Helicobacter pylori*-induced gastric carcinogenesis, which is currently being established in the group of Anne Müller at the IMCR. This model system recapitulates the progression of gastric cancer and could provide us with fresh tissue samples from pre-cancerous lesions for the investigation of DNA replication. Furthermore, we will test the possibility to perform EM analysis of replicating DNA from frozen or fixed tissue samples representing different stages of colon carcinogenesis, relying on tissue samples provided by Giancarlo Marra's group at the IMCR.

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

Arnab Ray Chaudhuri

Topoisomerase I (TopI) activity needs to be tightly regulated in every unperturbed cell cycle to ensure proper DNA replication fork progression and avoid DNA damage. TopI 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 TopI inhibitor (Camptothecin, CPT) was identified more than 30 years ago, recent water-soluble derivatives (topotecan and irinotecan) have been approved by the 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 TopI 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 double stranded-breaks (DSB) have been consistently proposed to mediate this cytotoxicity, several recent reports challenge this view and propose a more complex coordination of replication fork progression in face of the topological stress induced by TopI inhibition. Therefore, in order to gain mechanistic insights into genomic instability induced by

TopI inhibition in normal and cancer cells, further structural investigation is required.
We plan to investigate replication fork architecture and progression by a variety of structural and biochemical approaches (flow cytometry, bi-dimensional DNA electrophoresis, DNA fibre labelling, electron microscopy in combination with *in vivo*-psoralen crosslinking) applied to yeast and mammalian cells, as well as to *Xenopus* egg extracts, treated with specific TopI inhibitors. While fork progression will be studied by different approaches in yeast and mammalian cells, the detailed structure of replication intermediates will be directly visualized in all systems by electron microscopy, which will therefore be adapted to the study of DNA replication in *Xenopus* extracts, in collaboration with Vincenzo Costanzo's lab at Cancer Research UK. We aim to directly visualize how nucleosome displacement, template unwinding and DNA synthesis are modulated at DNA replication forks challenged by CPT and other TopI inhibitors.
By different genetic and biochemical tools (yeast mutants, siRNA in mammalian cells, immunodepletion in *Xenopus* extracts) we will then 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

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 related to DNA replication. A growing number of human neurological hereditary diseases, among which Huntington disease, myotonic dystrophy and fragile-X are the most prominent, have been associated with TNR expansion at their respective 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. These expansions take place during transmission from generation to generation and 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, but compelling evidence is still missing that those structures indeed form in living cells and contribute to TNR instability during DNA replication.
Taking advantage of TNR-containing plasmids, bi-dimensional DNA electrophoresis and electron microscopy in combination with *in vivo*-psoralen crosslinking, we aim to identify possible abnormal structures arising at different TNR sequences during *in vivo* DNA replication in cultured mammalian cells and to characterize their fine architecture. We will then test the role of candidate mammalian factors in the stability of these tracts during replication. By a combination of molecular and structural biological approaches, we also aim to isolate the abnormal DNA structures and use them to generate specific antibodies, in collaboration with the specialized group of Toshio Mori in Japan. By this means, we plan to obtain a simple read-out of genomic instability arising at replicating chromosomes and, as a long-term goal, extend it to genome-wide screen in mammalian cells, in an attempt to identify novel mammalian factors actively preventing genome instability during DNA replication.

These projects are funded by the SNF-professorship grant to Massimo Lopes and by the Start-up funding from the University of Zürich.

Giancarlo Marra

Colon cancer

Aberrant activation of the Wnt signaling pathway that controls epithelial cell division and migration, and defects in mismatch repair that is responsible for the correction of DNA replication errors have been implicated 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.

Postdocs
Mirco Menigatti
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PhD Students
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Amit Tiwari

Undergraduate students
Noemi Staehli
Mirjam Staubli

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 dinucleotide 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, Mirjam Staubli

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.

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 recruit-

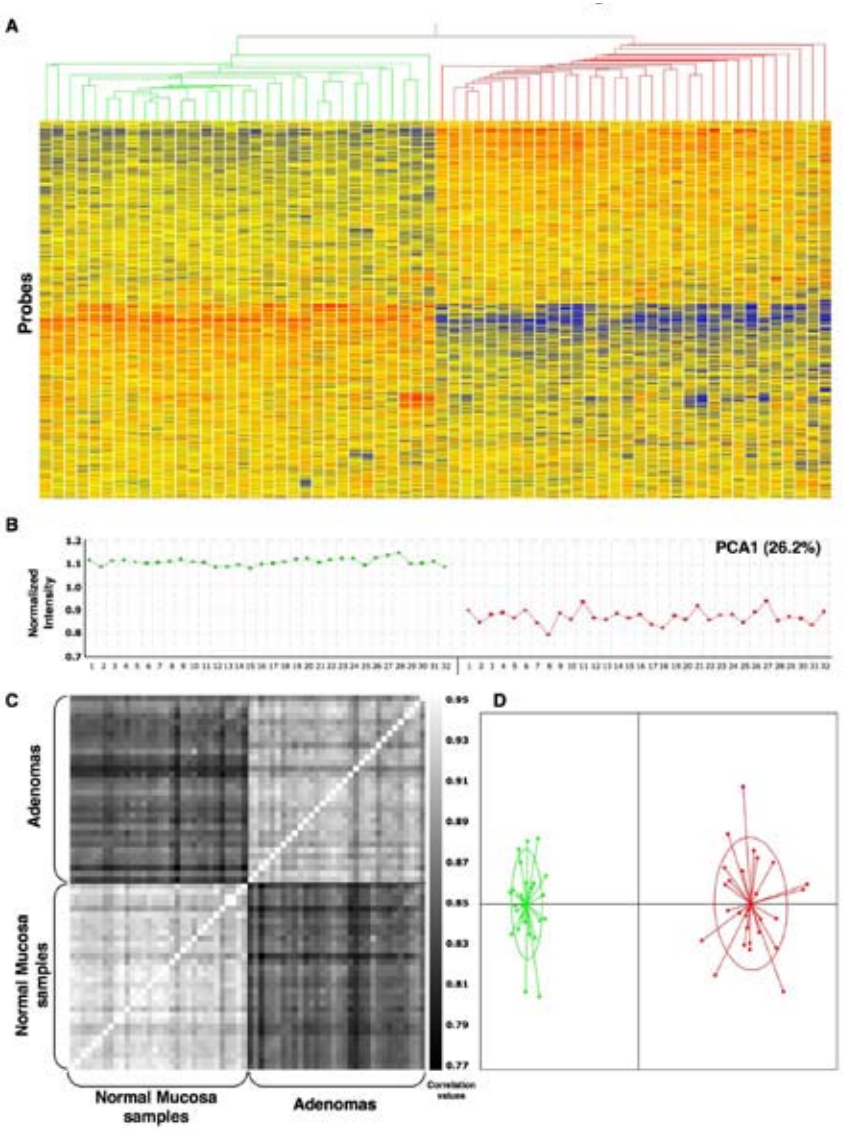

Figure 1.
The transcriptome of colorectal adenomatous polyps differs significantly from that of the normal colorectal mucosa (unsupervised analyses of microarray data).
A) Hierarchical clustering analysis. 64 tissue samples represented on the *x*-axis include 32 normal mucosal samples (green branches) 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.

ment 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 Karl Heinimann and his group. 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.

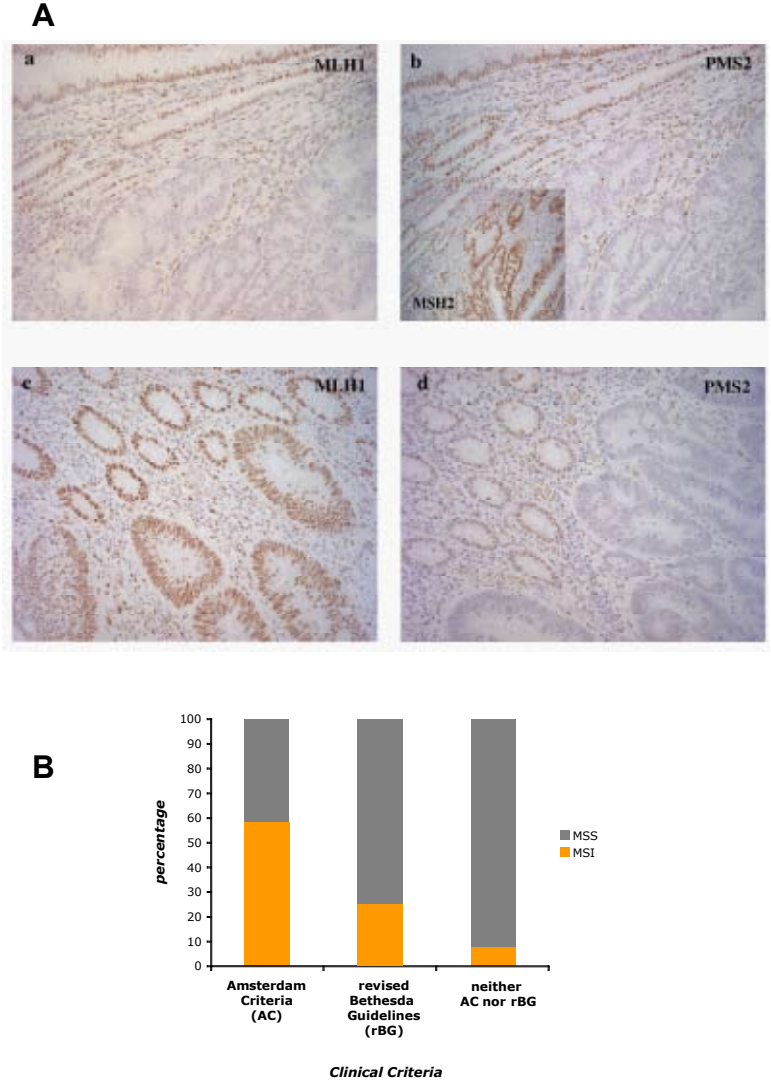

Figure 2.
Colon cancers with defects of the DNA mismatch repair.
A) Immunohistochemical staining of colorectal tumors for MMR proteins. Panels a and b: A tumor with a primary defect of the mismatch repair protein MLH1. a) MLH1 is absent from tumor tissue, but normal crypts (upper part of the picture) 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.

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

Emanule Valtorta, Noemi Staehli, Ritva Haider

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

In our immunohistochemical screening (see Figure 2), 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.

Genomic, epigenomic, transcriptomic and proteomic alterations caused by cigarette smoke in human bronchial epithelial cells

Amit Tiwari, Mirco Menigatti, Emilija Veljkovic

Molecular alterations in lung cancers of smokers may well be different from those present in lung cancers of non-smokers. Recently, "omics" studies have started to investigate this difference, and a large amount of data is being gathered. However, due to the heterogeneicity and complexity of the different types of lung cancers and even of single neoplasias, it is difficult to identify the molecular alterations that have a causal relationship with exposure to carcinogens present in cigarette smoke. We have initiated a study aimed at the identification of molecular alterations caused by cigarette smoke during initiation and progression of lung cancer. First, non-tumorigenic, human bronchial epithelial cells will be exposed to different regimens of cigarette smoke in order to select for surviving clones that will then be injected into mice for a further stage of selection. These consecutive selection steps should lead to the acquisition of a relatively homogeneous cell population, in which consistently-detected molecular alterations could be ascribed directly to the exposure to cigarette smoke. Second, lung cancers will be screened for such alterations, with the objective to identify those changes that specifically occur in cancers of cigarette smokers.

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Anne Müller

Helicobacter pylori and gastric cancer

Our laboratory is interested in elucidating the mechanisms linking chronic inflammation to carcinogenesis. Our model system is the infectious agent *Helicobacter pylori*, which persistently colonizes the human stomach and causes gastritis, ulcers and gastric cancer.

We use cell culture and animal models to better understand how the immune response to the infection, but also bacterial virulence factors and host factors such as gender and age at time of infection influence disease outcome.

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Helicobacter pylori and gastric cancer

Ayça Sayi, Isabelle Arnold, Iris Hitzler, Isabella Toller, Esther Kohler

In collaboration with Stanley Falkow, Dept. of Micorbiology and Immunology, Stanford University, USA and Joerg Huelsken, ISREC, Switzerland

My laboratory studies the interaction of the bacterial pathogen *Helicobacter pylori* with its host using a variety of *in vitro* and *in vivo* model systems. *H. pylori* persistently colonizes the human gastric mucosa; this process can lead to gastric alterations such as chronic gastritis and ulcers as well as two malignancies, gastric adenocarcinoma and B-cell lymphoma. We have established two laboratory mouse models that faithfully reproduce the progression of the human tumors via distinct precursor lesions; these models differ substantially in gender, genetic background and consequently in the type of immune response they launch against the infection. In addition, we use cell culture models- both cell lines and primary gastric epithelial cells- that allow us to study early aspects of *H. pylori*/host interactions as well as mechanisms of innate immune recognition. We use a variety of tools, ranging from immunofluorescence microscopy, immunohistochemistry, and FACS, to all standard molecular biology techniques involving protein, RNA and DNA analysis. A special emphasis of the lab is on genome-wide approaches such as expression profiling of purified cell populations obtained by laser-assisted microdissection. With this latter approach we hope to characterize the progenitor cell that gives rise to gastric adenocarcinomas, and identify key transforming events. Another major project focuses on the effect of *H. pylori* on cell cycle control and DNA mismatch repair. Our initial observation instigating this project was that infection of a gastric epithelial cell line with wild type bacteria completely blocked expression of the host cell's mismatch repair machinery. Interestingly, this effect was dependent on proper assembly of a functional type IV secretory system, which the bacteria utilize to translocate at least one effector molecule into the host cell's cytosol. Finally, we study the mechanisms of recognition of *H. pylori* by the host's innate immune system. To this end, we are currently using a large panel of k/o mice lacking one or more components of the innate and adaptive immune systems that will allow us to understand how these act together to cause the chronic inflammation and associated epithelial changes that are a hallmark of *H. pylori* infection.

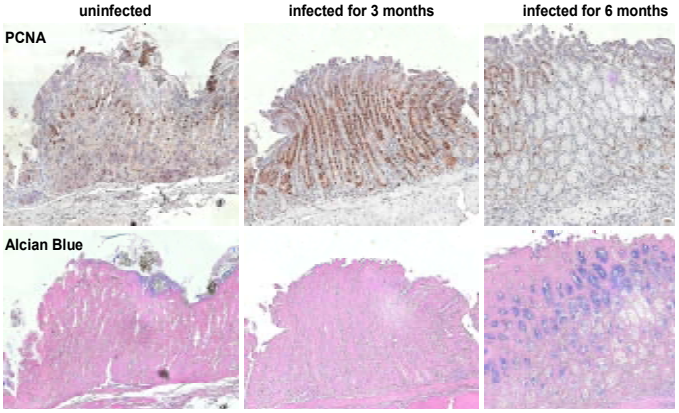


Figure 1.
Histopathology of a control mouse and two infected mice (3 and 6 months, respectively. Paraffin sections were stained with an antibody against the proliferation marker PCNA (upper panel) or with Alcian Blue dye, which stains the acidic, sulfated mucins typical of intestinal mucus cells.

Molecular pathogenesis of *Helicobacter*-induced mucosa-associated lymphoid tissue lymphoma in a mouse model

Isabelle Arnold, Vanessa Craig

In collaboration with Isidore Lossos, University of Miami, USA and Christiane Gerke and Stanley Falkow, Dept. of Microbiology and Immunology, Stanford University, USA

Mucosa-associated lymphoid tissue (MALT) lymphomas are B-cell lymphomas that arise at sites of chronic inflammation. Their normal cell counterpart is the memory B-cell of the marginal zone of secondary lymphoid organs. Gastric MALT lymphomas are closely linked to chronic infection with the bacterial pathogen *Helicobacter pylori*, which is typically acquired in childhood and normally persists for the lifetime of its host. Persistent *Helicobacter* infection results in chronic inflammation and accumulation of MALT, which provides the setting for outgrowth of neoplastic lymphomatous clones. We have developed and analyzed a BALB/c mouse model of the disease; in this model, MALT acquisition is observed after 15-18 months of infection and frank lymphomas are obtained after approximately 24 months. An animal study with 53 mice was initiated 1.5 years ago and will soon provide the fresh and cryopreserved material we need in order to address the following points. 1. The role of Th2-polarized T-cells in MALT lymphomagenesis *in vivo*. Late stage MALT lymphomas have typically acquired chromosomal translocations that allow them to grow autonomously. In contrast, early MALT lymphomas require antigenic stimulation, e.g. by *Helicobacter* antigen. Our previous studies have shown that murine MALT lymphomas are infiltrated by large numbers of Th2-polarized T-cells; we hypothesize that their cytokine products play an important role in inducing and maintaining tumor B-cell proliferation. In addition to a group of wild type BALB/c mice, mice deficient for Th2 responses (IL-4- and IL-4 receptor knock out) were infected. All mice will be analyzed with regard to colonization densities and susceptibility to MALT lymphoma. Preliminary results obtained at 12 months post infection suggest that the knock-out strains are more susceptible to the infection, as they are colonized at much higher densities than wild type. 2. The role of Th2 cells and their cytokine products *in vitro*. In addition to these *in vivo* studies, we will grow explanted tumor cells in the presence of *Helicobacter* heat-inactivated whole cell lysate, which triggers their proliferation. Parallel *ex vivo* cultures will be depleted of various populations of T-cells or other tumor-infiltrating cell types or of their cytokine products by magnetic bead technology for identification of the mitogenic signals and their sources. An antagonistic anti-CD40 antibody will further be used to determine whether a direct interaction between B- and T-cells is necessary for B-cell proliferation. B-cell proliferation will be measured by 3H thymidine or BrdU incorporation. 3. The specificity of tumor-derived immunoglobulin. Both the *ex vivo* cultures and their supernatants will be harvested and cryopreserved. Ig-secreting cultures will be selected for testing of corresponding supernatants in immunohistochemistry and Western blot assays for auto-reactivity (using sections or lysates from the same stomachs they were isolated from) or *Helicobacter* reactivity (using *Helicobacter* lysates separated by one- or two-dimensional gel electrophoresis). The frozen cultures of supernatants with promising reactivity will be used for hybridoma generation and production of clonal antibodies; alternatively, the tumor cells will undergo single cell sorting followed by cloning of individual pairs of heavy and light chain genes and recombinant expression in 293 cells. The specificity of the antibodies produced by either method will subsequently be tested using the methods above. The molecular targets will be identified by biochemical and proteomic approaches that are available to us through our Functional Genomics Center.

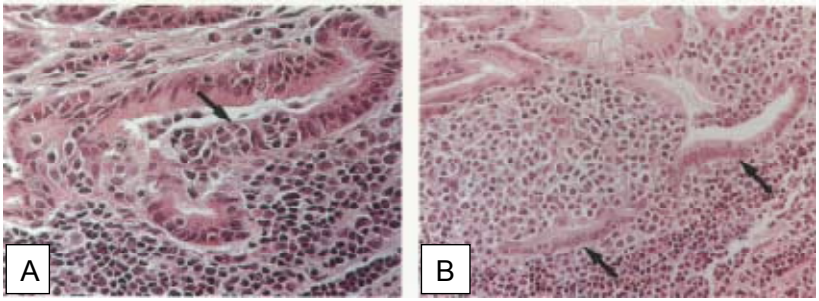
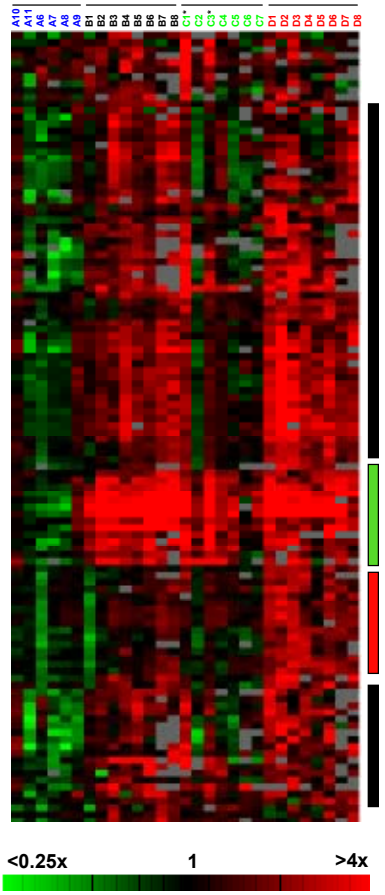


Figure 2.
Histological features of MALT lymphoma-like lesions in mice. A, Small aggregates of centrocyte-like cells (arrows) infiltrate hyperplastic foveolar epithelium in this relatively early lymphoepithelial lesion (H&E; original magnification, x 100). B, Fully developed LE lesion showing destruction of glandular and foveolar epithelium with a central epithelial remnant (arrows) (H&E; original magnification, x 63). From: Enno, A. et al., 1995.



A: control group (uninfected, age-matched)
B: infected for 24 months
C: infected; triple therapy-treated (* animals did not respond to therapy)
D: infected; triple therapy-treated; re-infected

surface molecules and signalling genes:
CD52, CD53, clusterin, Rho-GDI, protein kinase C, Bcl2-A1a, Bcl2-A1c ,Bcl2-A1d, immunoglobulin λ chain

MHC genes:
MHC class II antigens A and E, β-2 microglobulin, IAα, CD73

gene clusters indicative of present infection (MALT pathology)

B-cell signature genes:
immunoglobulin α and joining chain, CD40, granulin, integrin β7, GATA binding protein 2

gene cluster indicative of past or present infection

re-infection specific genes:
proliferation-associated gene A, ferritin heavy chain, glutathione S-transferase, SNF-1 like kinase

gene cluster indicative of re-infection

mucosal defense cluster:
small proline rich protein Sprr, secretory granule proteoglycan, immunoglobulin heavy chain, CDC-like kinase 3, small inducible cytokines

Figure 3.
Transcriptional profiling reveals clusters of genes whose expression patterns correlate with treatment outcome. RNA was prepared from whole stomach tissue of several animals per treatment group, reverse transcribed, and hybridized to 38,000 element murine cDNA arrays. Data were filtered with respect to spot quality and data distribution before clustering of the resulting 125 genes. Only genes for which information was available for >70% of arrays were included. Clusters of genes that are differentially regulated between treatment groups are annotated, and representative genes are listed for every cluster. From: Mueller, A. et al., 2005, Am. J. Path., 167: 797-812.

The role of Cox-2, PGE2 and β-catenin in *Helicobacter*-induced gastric cancer

Isabella Toller

Several tumor suppressor genes and tumor promoting signaling pathways have been implicated in the formation of gastro-intestinal tumors. We plan to focus on two of these in an attempt to clarify their role in *Helicobacter*-induced gastric carcinogenesis.
Individuals with a long history of low dose treatment with non-steroidal anti-inflammatory drugs have a significantly lower risk of developing various solid tumors. These drugs target cyclooxygenase-2 (Cox-2), a key enzyme in the prostaglandin E2 (PGE2) synthesis pathway. Once produced, PGE2 may act in a paracrine manner to induce pre-neoplastic changes in nearby cells, or in an autocrine manner on the cell that produced it. We will initially conduct three simple experiments to assess whether Cox-2 is at least in part responsible for the pre-neoplastic changes we observe in our model. First, we will assess whether Cox-2 is induced upon infection in a gastric epithelial cell line, in primary epithelial cells, and in our mouse model of *Helicobacter* infection. Preliminary data suggest that this is indeed the case. We will further treat infected mice with a Cox-2 inhibitor such as celecoxib in an attempt to block (or even revert) precancerous changes in the gastric epithelium. If this treatment is successful and Cox-2 therefore can indeed be implicated in *Helicobacter*-mediated neoplastic changes, we will perform the opposite experiment and supplement the murine diet with PGE2. This treatment should accelerate progression to gastric cancer (and might even substitute for infection as the key tumor promoting event). In subsequent experiments, we could use a commercially available Cox-2 k/o strain to further test the role of this enzyme in gastric carcinogenesis. Both heterozygous and homozygous mutant mice can be used; the latter have the clear disadvantage that 60% die *in utero* or as neonates and the average lifespan of surviving mice is only 3.5 months. While this should in principle be sufficiently long to see if lack of Cox-2 in the stomach can prevent precancerous lesions, we are aware of the fact that any real effects could possibly be masked by other health problems. We and others have shown before that β-catenin is stabilized in response to *H. pylori* infection of cultured epithelial cells and translocates to the nucleus where it activates transcription of a number of genes, some of which are implicated in gastric carcinogenesis (El-Etr et al., 2004; Franco et al., 2005). *H. pylori*-induced constitutive β-catenin activation has been suggested to promote the formation of pre-cancerous and cancerous lesions in humans and cancer in rodents - however the data at this stage is only correlative. In order to definitively assess the contribution of β-catenin to *Helicobacter*-induced carcinogenesis, we plan to use two genetically modified mouse models: the APCmin/+ mouse, which harbors a truncating deletion of the *apc* gene, as well as a tissue specific β-catenin k/o. The APCmin/+ mouse is prone to develop multiple adenomas of the small intestine, due to constitutive β-catenin signaling in progenitor cells of the intestinal villus. We know from our own and other investigators' work that Wnt signaling plays a similar role in stem cell maintenance in the stomach as it does in the intestine; therefore, we expect *Helicobacter*-induced tumor progression to be accelerated in this model. The tissue specific β-catenin k/o will represent the reversed situation. It will be generated by crossing two strains that will both be provided by collaborators: a strain homozygous for a "floxed" β-catenin allele and a strain that expresses Cre recombinase under the control of a stomach-specific promoter (Foxa3). The former has previously been used to generate multiple tissue specific k/o of β-catenin and the latter has successfully been used to direct tissue-specific gene ablation (of a transcription factor critical for normal gastric epithelial homeostasis, Klf-4) to the glandular gastric mucosa. If β-catenin signaling plays an important role in *Helicobacter*-induced cancer, the rate of cancer initiation and progression will be slower in β-catenin-ablated mice than their littermates or cancer formation will be prevented entirely.

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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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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), 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.

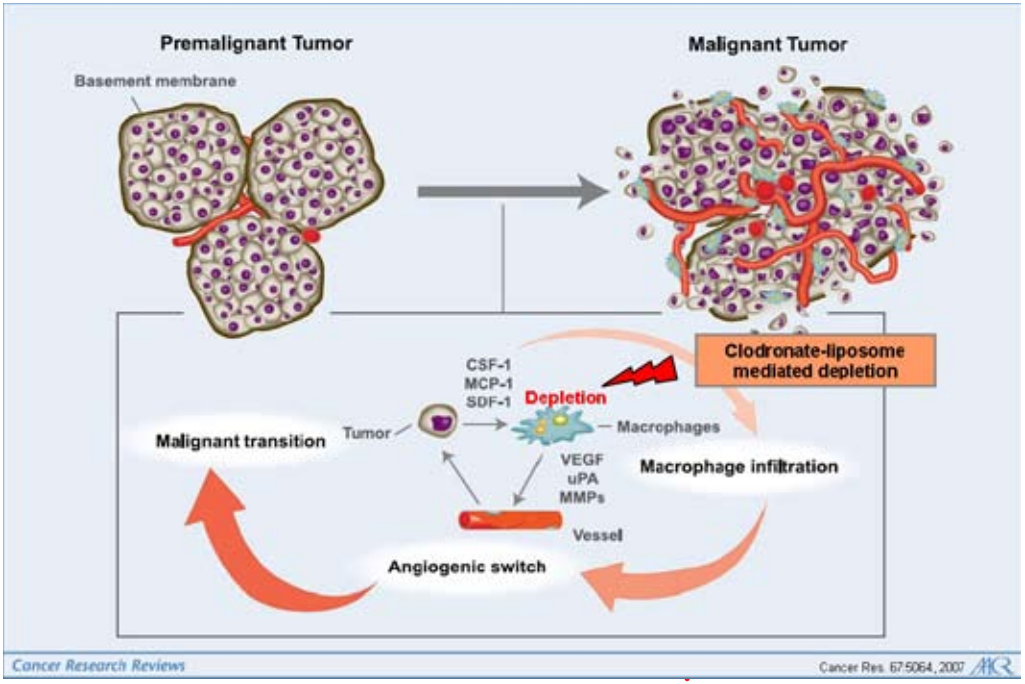

Figure 1.
Macrophages promote tumor progression through stimulation of the angiogenic switch. Depletion with clodronate-liposomes inhibits angiogenesis and other tumorigenic processes.

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

Sushil Kumar

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. Moreover, we will also exploit our co-culture models to identify nascent proteins translated in cancer and endothelial cells in response to macrophages. We will use a newly-developed mass spectrometry approach that utilizes bio-orthogonal analogs of amino acids to tag newly translated proteins, which can be used for their purification by affinity chromatography. These proteins can subsequently be identified by mass spectrometry. Significant molecular changes will be functionally characterized in co-culture assays. 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 by mass spectrometry, 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.

Gene expression study of macrophage depleted tumors

Anne-Katrin Bonde

For a preliminary gene expression study F9 teratocarcinoma tumors were grown in SV129S1 mice. The mice were either treated with clodronate-liposomes or left untreated. A gene expression experiment was done with the Affimetrix global mouse gene chip. Among many other genes affected by macrophage depletion, the data from the two groups confirmed changes of macrophage related genes, such as a 6 fold down-regulation of CSF-1 as well as an overall reduction of inflammatory responses. Moreover, the data suggested an overall down-regulation of the hem-angiogenic pathway and of factors known to be important for maintenance of the extracellular matrix, which was further confirmed by immunohistochemistry. To identify the cell types responsible for production of pro-carcinogenic cytokines and chemokines, and to identify signaling molecules produced by TAMs in particular, tumors generated *in vivo* are further analyzed for signaling and cell composition. The tumor stroma is examined to reveal macrophage effects on cell composition in developing tumors.
To further characterize these effects, *in vitro* migration assays with co-cultures of tumor cells and macrophages are used to identify components that stimulate cancer cells to display an invasive phenotype. Soluble factors (cytokines, chemokines) isolated from these co-cultures are analyzed by mass-spectrometry.

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

Sibel Mete

To elucidate the roles of TAMs in metastatic dissemination, we established orthotopic murine melanoma models. Highly metastasizing B16 melanoma cells were orthotopically injected into the ears or footpads of mice. Metastases were 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. Injection of labeled B16 cells into transgenic mice expressing EGFP in macrophages (Macgreen mice) allows whole body *in vivo* imaging of tumor growth and metastatic dissemination, as well as the study of migration of macrophages to metastatic niches. Results obtained from the *in vivo* imaging 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.

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

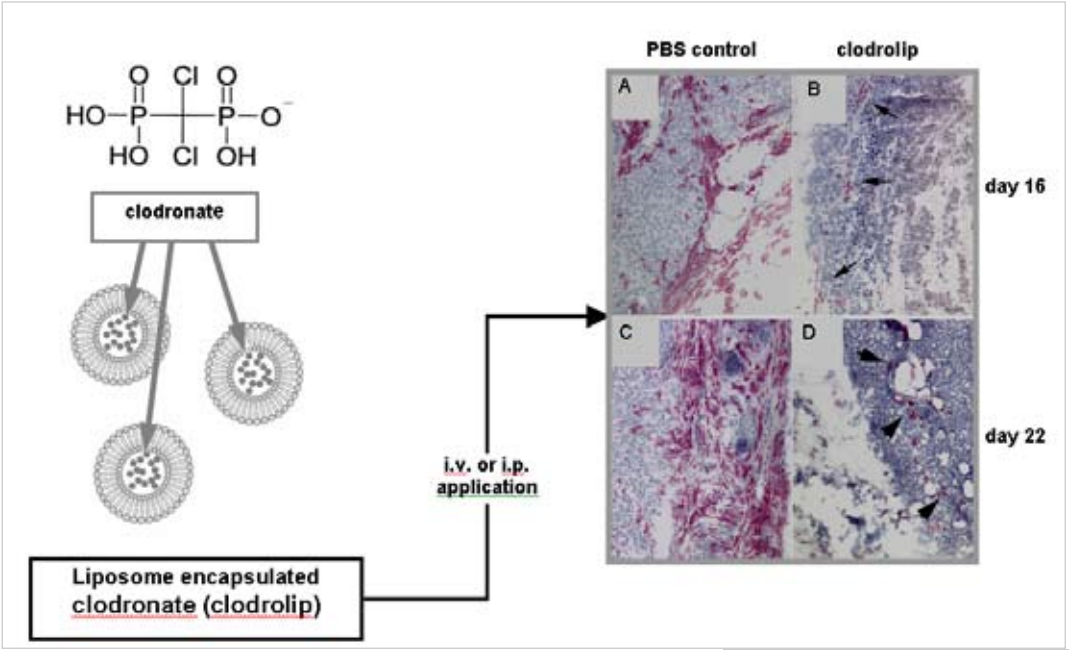

Figure 2.
Clodronate-liposome mediated depletion of TAMs in the A673 rhabdomyosarcoma model. Scheme of clodronate encapsulation (left), and immunohistochemical staining with the macrophage specific F4/80 antibody (right, from Br. J. Cancer 95: 272, 2006).

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.

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Book Chapters

Sabates-Bellver J., Cattaneo E., Heinimann K., Jiricny J., and Marra G. "Getting familiar with familial colon cancer". Chapter in "Intestinal inflammation and colorectal cancer". Ed. C. Gasche, JM Herrerias-Gutierrez, M. Gassuli, E. Monterio. Springer and Falk Foundation, pp 27-60.

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Collaborations

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Case Western Reserve University, Cleveland, USA
Cancer Research UK, London Research Institute, UK
Cantonal Hospital Lucerne, Switzerland
Centre Europeen pour la Recherche en Biologie et Medecine, France
CRC laboratories, UK
Danish Cancer Society, Denmark
École Polytechnique Fédérale de Lausanne, Switzerland
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ETH Zurich, Switzerland
Friedrich Miescher Institute, Basel, Switzerland
Harvard Medical School, Cambridge, USA
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
Korea Advanced Institute of Science and Technology, Republic of Korea
KuDOS Pharmaceutical Limited, UK
Leiden University Medical Center, The Netherlands
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Massachusetts Institute of Technology, Cambridge, USA
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National University of Ireland, Galway, Ireland
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Universität Köln, Germany
University of Basel, Switzerland
University of California, USA
University of Cambridge, UK
University of Dundee, Scotland, UK
University of Edinburgh, UK
University of Geneva, Switzerland
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 Ulm, Germany
Vanderbilt University, Tennessee, USA
Washington University School of Medicine, USA
Wayne State University, Detroit, USA
Yale University, New Haven, USA

Teaching

Josef Jiricny and Anne Müller
Basic Molecular Biology
Biochemistry I, for medics, University of Zurich

Josef Jiricny and colleagues
Radiobiological Seminars
DNA Damage Repair and Cancer
Repair, Replication and Recombination Club, University of Zurich

Stefano Ferrari
Ph.D. Program "Biochemistry and Biotechnology" Lecture Series, Faculty of Medicine, University of Padua, Padua, Italy
Workshop on post-translational modifications of proteins, UZH

Giancarlo Marra
EU Integrated Program DNA damage response and repair mechanisms, April 2006, Trondheim, Norway
Molecular Biology in Oncology tutorials for students at the Faculty of Medicine of the University of Zurich
Clinical Oncology tutorials for students at the Faculty of Biological Sciences (Biomodul 415) of the University of Zurich
Mantelstudium Oncology SS07 lessons at the University of Zurich
RRR (DNA Replication, Repair, and Recombination) Club Meeting at the Institute of Molecular Cancer Research of the University of Zurich
Intensive Course of Molecular Medicine, University of Oslo, Norway
Clinical Oncology tutorials for students at the Faculty of Biological Sciences (Biomodul 415) of the University of Zurich
Mantelstudium Biomedizinische Wissenschaften SS07 at the UZH
Molecular Mechanisms of Host Defence" EU course organized by the Norwegian University of Science and Technology, Norway

Reto Schwendener
Project oriented learning (POL) course at the Faculty of Medicine, University of Zurich

Conferences

IMCR leaders were invited to present seminars at the following venues:

Stefano Ferrari
Novartis Pharmaceuticals, Basel, Switzerland
USZ Interdisciplinary Seminars, Dept. of Gynecology, Zurich, Switzerland
11th World Congress on Advances in Oncology and 9th International Symposium on Molecular Medicine, Hersonissos, Crete, Greece
4th MYB workshop: MYB proteins in Death, Differentiation and Disease, Civitella Alfedena, Italy

Pavel Janscak
Institute of Molecular Genetics AS CR, Prague, Czech Republic
Institute of Physical Biology, University of South Bohemia, Nove Hrady, Czech Republic
Weatherall Institute of Molecular Medicine, University of Oxford, UK
RecQ Helicases and Other Helicases in Telomere Maintenance and Related Pathways, National Conference Center, Lansdowne, Virginia, USA

Josef Jiricny
International FEBS Meeting, Istanbul, Turkey
GRC on Mutagenesis, Salve Regina, Rhode Island, USA
Erling Seeberg Symposium on DNA Repair, Lofoten, Norway
DNA repair: from molecular mechanism to human disease, Noordwijkerhout, The Netherlands
Basel Oncology Day, Basel, Switzerland
Cancer 2006: from molecular biology processes to tumor-tailored therapy, Stara Lesna, Slovak Republic
Beatson Int. Cancer Conference, Glasgow, UK
EEMS Meeting, Basel/Zurich, Switzerland
Rijkshospitalet, Oslo, Norway

Massimo Lopes
3rd Swiss meeting on genome stability, DNA dynamics and epigenetics, Uetendorf, Switzerland
MGMT and alkylating drug resistance, Mainz, Germany

Giancarlo Marra
University of Catanzaro, Italy
Institute Humanitas Milano, Italy
Falk Symposium 158 on Intestinal Inflammation and Colorectal Cancer, Seville, Spain
Spring Meeting of the Swiss Society of Medical Genetics, Zurich, Switzerl.
Oncology Institute of Southern Switzerland, Bellinzona, Switzerland
University Hospital, Zurich, Switzerland
Annual meeting of the Italian Association for the Study of Hereditary Gastrointestinal Tumors, San Giovanni Rotondo, Italy

Anne Müller
Annual conference of the Swiss Society for Infectious Diseases, Zurich
Helicobacter workshop, German Society for Hygiene and Microbiology, Herrsching am Ammersee, Germany

Reto Schwendener
Bayer Schering Pharma AG, Therapeutic Research Group Oncology, Berlin, Germany
Swiss Institute for NBC Protection, Spiez, Switzerland

Awards

Josef Jiricny
International Prize of the Slovak Academy of Sciences for outstanding work in the field of natural sciences
Fondazione San Salvatore Award 2005 for his contribution to cancer research.

Giancarlo Marra
50-year Jubilee Award for Cancer Research by the Zurich Cancer League

Massimo Lopes
Ernst Th. Jucker Prize 2006

Seminars 2006

Programmed cell death: can't live without it !
Christoph Borner, Institute of Molecular Medicine and Cell Research, University of Freiburg, Germany

Investigating the function and composition of human kinetochores
Patrick Meraldi, ETH Zurich, Switzerland

Regulating the fidelity of cell cycle events
Viesturs Simanis, ISREC, Epalinges, Switzerland

Somatic hypermutation of immunoglobulin genes
Ursula Storb, University of Chicago, USA

Chromatin assembly factors: histone H3 variants and cell cycle
Genevieve Almouzni, Institut Curie - Recherche, Paris, France

Antiangiogenic drugs increase the efficacy of oncolytic viruses targeting colon cancer
Richard Iggo, Bute Medical School, University of St Andrews, UK

Repair of DNA damage generated by anticancer drugs
Tomas Lindahl, London Research Institute, South Mimms, UK

Human and bacterial hypermutation
Myron Goodman, University of South California, Los Angeles, USA

Human UPF1 and EST1/SMG6: Connecting RNA and DNA surveillance pathways?
Joachim Lingner, Swiss Federal Institute of Technology Lausanne (EPFL), Switzerland

Chromosomal single-strand break repair and human disease
Keith W. Caldecott, University of Sussex, UK

DNA translesion synthesis and mismatch repair: The Yin and Yang of DNA damage responses, Niels de Wind, Leiden University, The Netherlands

Visualization of DNA double strand break repair in live bacteria
Peter Graumann, University of Freiburg, Germany

Mechanisms preventing recombination at replication forks
Marco Foiani, IFOM - F.I.R.C. Institute of Molecular Oncology, Milan, Italy

Towards the structural visualization of genomic instability during DNA replication
Massimo Lopes, University of Zurich, Ernst Th. Jucker Prize 2006 Winner, Switzerland

Stroma irradiation promotes tumor invasion and metastasis by suppressing angiogenesis : paradigm or paradox?
Curzio Ruegg, Cancer Center Lausanne and ISREC/NCCR Molecular Oncology, Switzerland

Mammalian Cells Cycle with Fewer and Fewer Cdk's
Mariano Barbacid, Spanish National Cancer Research Centre, Madrid, Spain

Genomic instability and cancer: lessons from analysis of Bloom's syndrome
Ian Hickson, University of Oxford, UK

Role of ubiquitin and SUMO in DNA transactions
Stefan Jentsch, Max Planck Institute of Biochemistry, Munich, Germany

About signaling pathways, proteomics and diseases
Jan van Oostrum, Dept of Science and Technology, Novartis Pharma, Basel, Switzerland

DNA deamination in antibody diversification and immunity to retroviruses
Michael Neuberger, MRC Laboratory of Molecular Biology, Cambridge, UK

Seminars 2007

Genome integrity pathways: Mechanisms revealed by real-time imaging of protein dynamics
Jiri Lukas, Institute of Cancer Biology and Centre for Genotoxic Stress Research, Danish Cancer Society, Denmark

Notch: lineage specifier, oncogene, tumor suppressor, and stem cell gate keeper
Freddy Radtke, Swiss Institute for Experimental Cancer Research (ISREC), Epalinges, Switzerland

The histone acetyl-transferase Tip60 suppresses Myc-induced lymphomagenesis through an oncogene-induced DNA Damage Response
Bruno Amati, European Institute of Oncology, Milan, Italy

New Branches in the ATM-Mediated DNA Damage Response
Yossi Shiloh, Sackler School of Medicine, Tel Aviv University, Israel

Molecular Mechanisms of Immunoglobulin Gene Diversification: Ubiquitous Repair Factors in a Mutagenic Pathway
Nancy Maizels, University of Washington, USA

The ubiquitin ligase E6-AP and its role in human disease
Martin Scheffner, Dept. of Biology, University of Konstanz, Germany

Androgen receptor function is controled by a multiple-specificity histone demethylation complex
Roland Schüle, Molecular Gynaecology Research Group, Center for Cllinical Research, University of Freiburg, Germany

DNA damage response and genome stability: The role of ATM, ATR and the Mre11 complex
Vincenzo Costanzo, Genome Stability Unit - Clare Hall Laboratories, Cancer Research UK, UK

Links between BRCA1 and the Fanconi anaemia pathway for repair of DNA crosslinks
Kevin Hiom, MRC Laboratory of Molecular Biology, Cambridge, UK

From genes to proteins and back:....
Anna Tramontano, Department of Biochemical Sciences, University of Rome "La Sapienza", Italy

Systems Biology of PARPs and PARG
Guy Poirier, Centre de recherche du CHUL (CHUQ), Quebec City, Canada

A novel, Cul3-based E3-ligase is required for faithful mitosis in human cells
Izabela Sumara, Institute of Biochemistry, ETH Zurich, Switzerland

Damage recognition in nucleotide excision repair
Nora Goosen, Laboratory of Molecular Genetics, Leiden University, The Netherlands

Repair of chemotherapy-induced DNA damage; opportunities for modulating drug resistance and sensitivity
John J. Turchi, Dept. of Biochemistry and Mol. Biology, Indiana University, Indianapolis, US

Involvement of structure-specific endonucleases in chromosome stability
Katsuhiro Hanada, The Weatherall Institute of Molecular Medicine, University of Oxford, UK

Social life

IMCR Personnel

Principal Investigators

Stefano Ferrari
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Josef Jiricny
Massimo Lopes
Giancarlo Marra
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* left IMCR during 2006/07