imcr

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Introduction

Having consolidated its position among the leading European institutes of molecular cancer research, the IMCR will look back on the year 2004 as the end of an important chapter in its development. One of the facets that are inevitably linked with increased visibility is that the attractiveness of the scientific staff in the eyes of outside parties increases. Predictably, Dr. Primo Schär and Dr. Orlando Schärer were offered several attractive independent positions during the past twelve months. Following extensive negotiations with the University, they finally decided to pursue their respective academic careers outside of Zurich. Dr. Schär accepted an offer of Associate Professor of Molecular Genetics at the University of Basel, and has taken up his post during the summer of 2004. His research group transferred to the Institute of Biochemistry and Genetics, DKWB, at the same time. Dr. Schärer has been offered a similar post at Stony Brook University on Long Island, and will be moving, together with most of his group, in the summer of 2005. These departures represent a substantial loss to the institute, while, at the same time, opening new doors of opportunity. Dr. Schär's position could be upgraded to one of Assistant Professor, which should make it more attractive to his potential successor. In addition, a second Assistant Professor position (tenure track) could be created within the framework of the Systems Biology Initiative of the University. It is hoped that these posts will be occupied in the second half of 2005.

In keeping with the major change in the composition of the IMCR, the institute was re-housed in May 2005. Our new premises, on the main campus of the University at Irchel will permit better scientific exchange with the other Institutes, particularly those active in the areas of molecular biology and biochemistry. We shall also gain better access to the central facilities of the University. Most important will be our proximity to the Functional Genomics Center Zurich, given that many new projects in the research areas of the institute depend on access to the transcriptomics and proteomics facilities.

IMCR scientists were again successful in attracting substantial external support in terms of grants from the Association for International Cancer Research, Bonizzi-Theler Stiftung, European Molecular Biology Organisation, Hartmann-Müller Stiftung, Hauptabteilung für Sicherheit der Kernanlagen, Hermann Klaus-Stiftung, Human Frontier Science Program, Julius Müller-Stiftung, Krebsliga des Kantons Zurich, Novartis Foundation, Sassella Stiftung, Swiss Cancer Research League, Swiss National Science Foundation and UBS AG.

IMCR staff continued to teach both in undergraduate and postgraduate courses. It was involved in the organisation of internal and external courses of further education as well as in the organisation of two national congresses. Thanks to its external funding, the Institute has been able to increase the number of junior research scientist at the level of PhD students and postdoctoral assistants. One of the senior scientists of the IMCR is a START fellow, sponsored by the Swiss National Science Foundation.

IMCR scientists are involved in several collaborative projects, both formally and informally. In the TANDEM program of the Swiss National Science Foundation, we have been able to identify more than 300 Swiss families with predisposition to colon cancer. About a third of these are afflicted with Hereditary Non-Polyposis Colon Cancer (HNPCC) that is linked to inherited mutations in mismatch repair genes. The mutation carriers among these family members are enrolled in genetic counselling and endoscopic screening programs. IMCR scientists participated in two European Community research programs, one of which they co-ordinated.

IMCR staff published 23 peer-reviewed papers, 5 review articles, 3 editorials in high impact factor journals and one book chapter. Moreover, IMCR scientists are actively engaged as external reviewers or editors of many top journals in the field of molecular biology and biochemistry.

IMCR has now become established as the foremost centre of basic cancer research in Zurich. One of the past goals was to strengthen this discipline by catalysing the interactions with other research groups at the University and ETH. This was done in the first instance through the creation of the Repair, Replication and Recombination network, the so-called RRR-Club, which was founded in 1996 and which attracts more than 80 scientists to its monthly reunions. The creation of the Cancer Network Zurich (www.cnz.unizh.ch) is an attempt to establish closer links with the basic and the clinical cancer research communities through collaborations, information exchange and further education. The CNZ currently consist around 40 groups from the Zurich area. The strengthening of communications and information exchange between basic research scientists and clinicians is essential if high quality innovative research into malignant disease is to be ensured in the long term. One additional key activity of the past year concerns the conception of the University Research Priority Program in Systems Biology and Functional Genomics. It is envisaged that this activity will become an essential part of the SystemsX project, a joint initiative of the ETHZ and the Universities of Zurich and Basel. IMCR plans to play an important role in this program. One Assistant Professor (tenure track) linked with this initiative will be housed in the institute in the coming years.

Prof. Dr. Josef Jiricny

Director

DNA damage and the regulation of cell division

Dr. Stefano Ferrari

Dr. Mahmoud El-Shemerly
Dr. Elisabetta Pani
Agnieszka Krystyniak / PhD Student
Christiane Köenig / Technician

Studies conducted in recent years have exposed in great detail the mechanisms that control entry and progression through the cell division cycle. These studies have shown that positive (i.e., mitogenic) and negative (i.e., stress) stimuli deriving from the environment or originating from within the cell, directly signal to the machinery that controls key transitions in the cell cycle. Signal transduction within cells relies on rapid and reversible post-translational modification (PTM) of proteins. This includes phosphorylation, which is the most common PTM involved in essentially all aspects of cellular metabolism, poly-ubiquitinylation that has a primary role in protein degradation, mono-ubiquitinylation and sumoylation, which are involved in DNA repair, acetylation and methylation, which play a role in transcriptional regulation and chromatin remodelling, respectively.
The broad interest of my laboratory is on pathways triggered by DNA damage and impinging onto the cell cycle machinery. In particular, three aspects are currently under intense scrutiny: (i) the identification and role of post-translational modifications of proteins involved with mismatch repair; (ii) the investigation of cisplatin-induced DNA damage signalling pathways; (iii) the effect of DNA damage on the onset of mitosis.

Stefano Ferrari

Regulation of mismatch repair proteins by phosphorylation

Dr. Mahmoud El-Shemerly

The increased rate of mutation and the destabilization of microsatellites resulting from inactivation of MMR confers a strong mutator phenotype on tumour cells. This in turn results in mutations in multiple oncogenes and tumour suppressor genes during the lifetime of an individual. In this study we address the possibility that components of the mismatch machinery are regulated by phosphorylation.
The MMR component that we have initially examined, hEXO1, is an exonuclease belonging to a family of enzymes playing important roles in DNA synthesis, recombination, repair and apoptosis. We found that hEXO1 was constantly expressed throughout the cell cycle. However, in response to the administration of agents that stall replication, rapid hEXO1 degradation ensued and this effect was reversible. Degradation of hEXO1 was specific to S-phase and strictly linked to DNA synthesis inhibition. DNA damage caused by UV-C radiation, ionizing radiation, cisplatin, or the alkylating agent MNNG, did not affect hEXO1 stability. We obtained evidence indicating that hEXO1 was phosphorylated in response to inhibition of DNA synthesis and that phosphorylation coincided with rapid protein degradation through ubiquitin-proteasome pathways. Our data support the evidence that control of hEXO1 activity may be critical for the maintenance of stalled replication forks (Fig. 1).
A recent development of this study has led to the identification of the E3 ligase KAP1-MDM2 complex among the proteins interacting with hEXO1. Current effort aims at clarifying the role of KAP1-MDM2 in hEXO1 polyubiquitinylation and at reconstructing the hEXO ubiquitinylation machinery in vitro.

This study is supported by the SNF, the Sassella-Stiftung and the Ida-de-Pottere-Stiftung.

Signalling pathways tiggered by cisplatin

Dr. Elisabetta Pani

Platinum-based drugs are heavy metal complexes containing a central platinum atom and are widely used in the treatment of human malignancies. Despite their widespread use however, side effects as well as the acquisition of resistance seriously limit the deployment of platinum in the clinic. Aim of this study is to gain a deeper understanding of the mechanism of sensitivity to cisplatin in order to be able to target key pathways before the emergence of resistance.
At the molecular level, signalling of cisplatin-induced damage occurs following recognition of DNA distortions by a number of proteins, such as the MutSa component of the mismatch repair system, the high mobility group proteins HMG1 and HMG2, the RNA pol-I binding factor hUBF and the TATA-binding protein TBP. We have examined matched cell lines that display differential sensitivity to cisplatin. Interestingly, we observed that upon cisplatin treatment, sensitive cell lines remained fully arrested at the G2/M transition of the cell division cycle

and apoptosis ensued as result. At the molecular level this is accompanied by loss of proteins participating in homologous recombination, namely BRCA1, BRCA2, FANCD2 and RAD51.

On the contrary, cisplatin-resistant cells arrested only temporarily at G2/M, did not lose homologous recombination proteins and, after bypassing the block, resumed progression through mitosis and continued cycling. Permanent activation of DNA damage signalling pathways in the resistant cells indicated that they apparently re-entered the cell cycle with damaged DNA. In strictly isogenic cell lines (i.e., that are only differing for the expression of one of the components of the MMR system) we observed that MMR-proficient cells arrested in S-phase, whereas MMR-deficient cells arrested prior to mitosis. In both cases, however, the arrest was permanent and was followed by apoptosis, indicating that defects in MMR are not sufficient to explain the ability of cisplatin-resistant cells to bypass the DNA damage checkpoint.

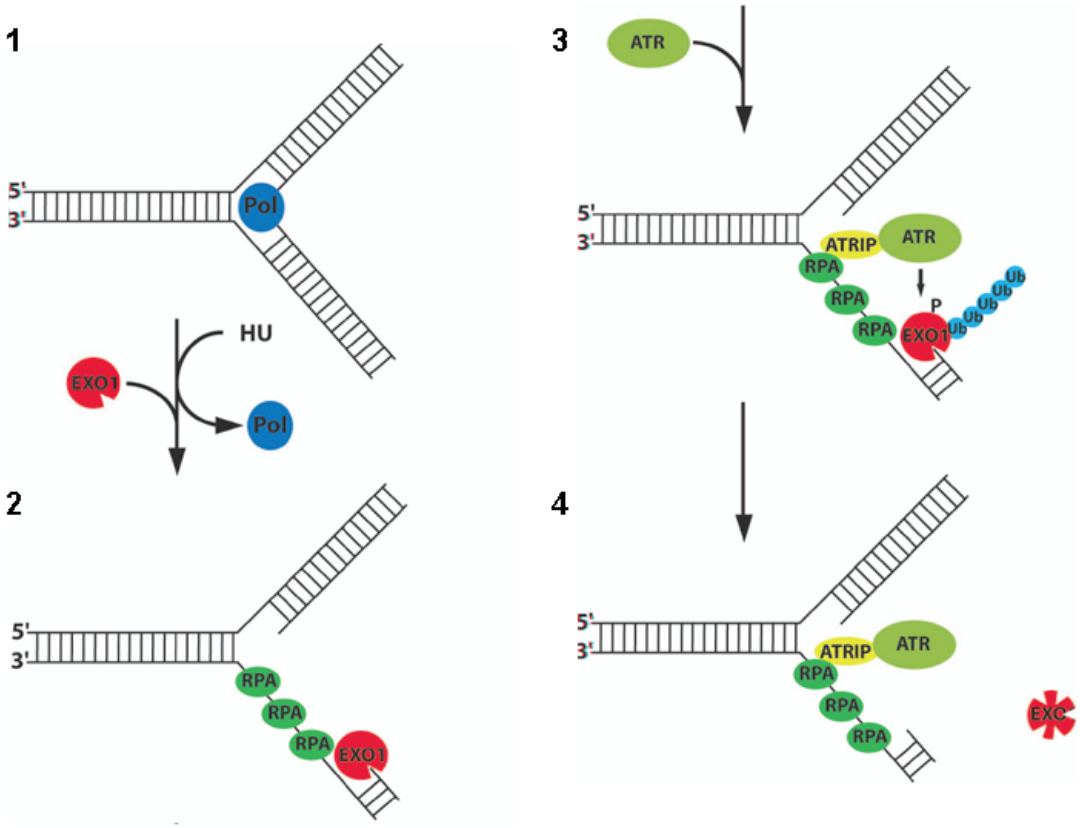

Figure 1: Hypothetical model of hEXO1 regulation at stalled replication forks.

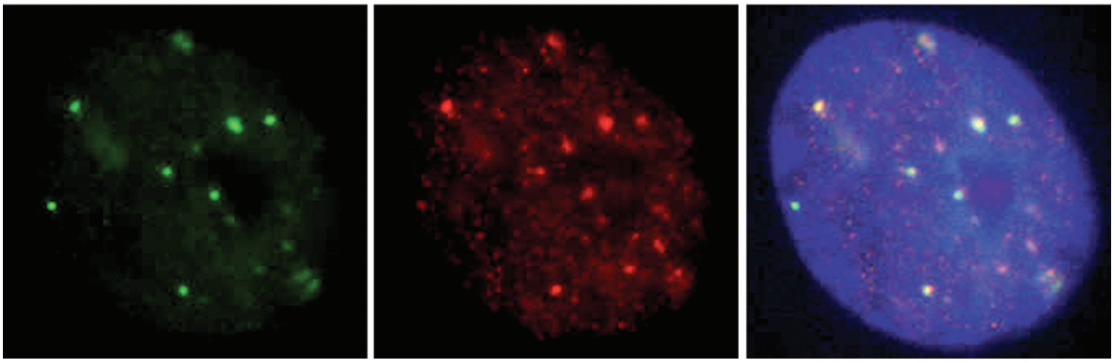

Figure 2: Immunostaining of cisplatin-resistant ovarian cancer cells (CP70) upon 24 hrs treatment with cisplatin. Green: RAD51; Red: BRCA1, Blue: DNA.

DNA damage and the regulation of mitosis

Agnieszka Krystyniak, Christiane König

Transition through mitosis is an obligatory 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. We have recently provided evidence that inhibition of the protein kinase Aurora A (AurA) is part of the DNA damage response. Using selective inhibitors and siRNA, we have shown that DNA damage-dependent inactivation of AurA is mediated through Chk1-dependent signalling and occurrs independently of Cdk1 inhibition. Analysis of AurA inhibition at the molecular level indicated that DNA damage results in release of protein phosphatase 1 (PP1) and that this effect is abolished in a mutant of a site of phosphorylation (Ser342>Ala) adjacent to the PP1 binding domain in AurA. Furthermore, we have addressed the impact of AurA kinase inhibition on mitotic entry. By means of transient transfections and protein transductions we have shown that constitutively-active AurA could bypass the DNA damage checkpoint and cause mitotic entry via reactivation of Cdk1. This indicates that AurA plays a key role upstream of Cdk1.
Building on this knowledge, we have initiated a new study aimed at exploring whether some cancer cell lines and tumours in vivo may be inherently more resistant to DNA damaging agents by virtue of their documented AurA overexpression.The study consists of first characterizing the extent of resistance of such cell lines to DNA damaging agents and then exploring the possibility that downregulation/inhibition of AurA (either by siRNA or by chemical compounds) may re-sensitize these cells to genotoxic therapy.
The gap between the growing evidence on the role that AurA plays in cancer on the one hand, and the limited amount of information on its physiological substrates on the other, prompted us to undertake a study aimed at examining the structural features determining AurA substrate specificity. We have obtained evidence that AurA recognizes the consensus sequence R/K/N-R-x-S/T-B, where B denotes any hydrophobic residue with the exception of Pro. This study, in turn, has provided the tools for prediction and testing of physiologically relevant AurA targets.

These projects are financed by the Zurich Cancer Research League, Novartis Pharma AG and the Lydia Hochstrasser-Stiftung.

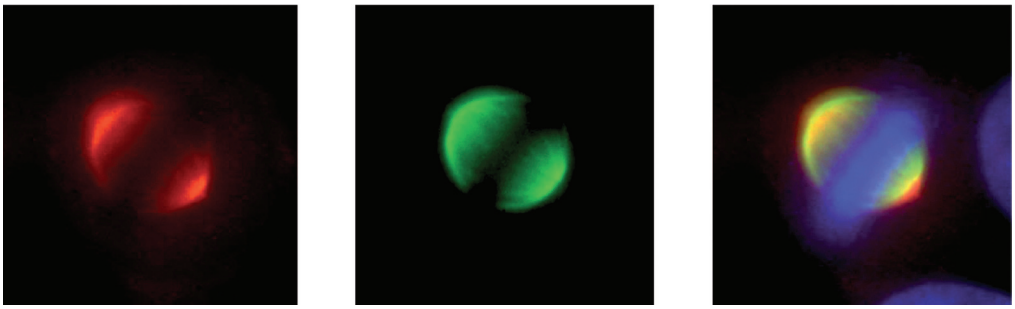

Figure 3: staining of mitotic HeLa cells. Blue: DNA; Green: AurA; Red: β-tubulin.

RecQ helicases and the maintenance of genomic stability

Dr. Pavel Janscak

Dr. Nurten Saydam
Patrick Garcia / PhD Student
0 Zheng Lu / PhD Student
Radhakrishnan Kanagaraj / PhD Student
Sybille Schwendener / PhD Student

We explore the biological processes that ensure genomic stability. The main objective of our research is to provide a clear understanding of the molecular mechanisms involved in the suppression of inappropriate DNA recombination events during DNA replication and repair that can cause chromosomal rearrangements and tumorigenesis.

Pavel Janscak

Role of the human RECQ5ß helicase in maintenance of genomic stability

Patrick Garcia, Zheng Lu, Radhakrishnan Kanagaraj

Homologous recombination (HR) provides an efficient and error-free pathway of repair of DNA double-strand breaks and single-strand gaps, especially in replicating cells where the undamaged sister chromatid can serve as a repair template. However, the HR machinery has to be tightly controlled to protect the genome from abortive recombination events that can lead to potentially lethal chromosomal rearrangements. The product of the human *RECQ5* gene, a member of the RecQ family of DNA helicases, has been implicated in the suppression of mitotic recombination, but its exact cellular role is not known. The RECQ5 protein exists in three different isoforms resulting from alternative RNA splicing. We have characterized the biochemical properties of the large splice variant, RECQ5β. These studies indicated that RECQ5β is an ATP-dependent 3'-5' DNA helicase possessing the ability to promote strand exchange and Holliday-junction branch migration. However, to promote efficient unwinding of oligonucleotide-based substrates, RECQ5β required a single-stranded DNA binding protein such as replication protein A (RPA). Surprisingly, we found that RECQ5β possesses an intrinsic DNA strand-annealing activity that is strongly inhibited by RPA and ATPγS, a poorly hydrolysable analog of ATP. The inhibitory effect of ATPγS was alleviated by mutations in the ATP-binding motif of RECQ5β, indicating that the ATP-bound form of the protein cannot promote strand annealing. Analysis of deletion variants of RECQ5β revealed that the DNA helicase activity resides in the conserved N-terminal portion of the protein, whereas the unique C-terminal domain mediates strand annealing. This work was the first demonstration of a DNA helicase with an intrinsic DNA strand-annealing function and suggested that RECQ5β could be involved in DNA transactions that require the coordinated action of a helicase and strand-annealing activities.

More recently, we have obtained evidence suggesting that the RECQ5β protein is localized in the DNA replication factories in S-phase nuclei and persists at the sites of stalled replication forks (Figure 1). RECQ5β was also found to localize to the sites of DNA double-strand breaks (DSBs) and to physically interact with the MRE11 complex that is involved in DSB repair. Moreover, we found that RECQ5β physically interacts with the RAD51 protein, a key HR factor that promotes homologous DNA pairing and strand exchange. We have also found that RECQ5β directly interacts with SKP1, the scaffold component of SCF ubiquitin ligases, of which one, named SCF^{FBH}, has been shown to be involved in the anti-recombination process in yeast. The functional significance of these protein-protein interactions is currently being investigated.

This project is funded by the Cancer League of the Canton Zurich, the Swiss National Science Foundation and the Sassella Stiftung. It was carried out in collaboration with Prof. Ian D. Hickson, University of Oxford and Dr. Stephen C. West, Cancer Research UK.

Mechanismus involved in the suppression of homeologous recombination

Nurten Saydam, Patrick Garcia

Homeologous recombination that occurs between DNA sites of imperfect homology can give raise to detrimental chromosomal rearrangements. The DNA mismatch repair (MMR) system has been implicated in the suppression of this kind of DNA recombination, but the underlying mechanism remains elusive. Heteroduplex rejection during repair of DSBs by the single-strand annealing pathway of homologous recombination in yeast requires the mismatch binding and ATPase functions of the Msh2p/Msh6p heterodimer and the helicase activity of Sgs1, suggesting a model in which the MMR proteins act in conjunction with a RecQ helicase to unwind DNA

recombination intermediates containing mismatches. Consistent with this hypothesis, we found that the MSH2/MSH6 (MutSα) and MSH2/MSH3 (MutSβ) heterodimers, significantly stimulated the DNA helicase activity of the human Sgs1 ortholog WRN whose deficiency is known to be associated with elevated levels of chromosomal rearrangements and cancer predisposition. Moreover, we demonstrated that WRN interacts with MutSα and MutSβ physically, suggesting that unwinding of recombination intermediates by WRN is facilitated through interaction with bound MMR proteins.
We also found WRN to physically interact with the MLH1/PMS2 (MutLα) heterodimer that, in the mismatch-repair pathway, serves as matchmaker, which binds to the mismatch recognition complex and mediates the recruitment of downstream factors. However, MutLα did not enhance the stimulatory effect of MutSα and MutSβ on WRN-mediated DNA unwinding *in vitro*, confirming the yeast genetic data indicating that the Mlh1 and Pms1 proteins have only minor roles in the rejection of homeologous recombination. Nevertheless, we have found that WRN forms a complex with MLH1 and PMS2 *in vivo*, specifically after exposure of cells to ionizing radiation that can induce DSBs. Moreover we have found that MLH1 is a part of PML bodies and co-localizes with WRN at distinct nuclear foci after ionizing radiation. Thus, it is plausible that MLH1 is involved in the recruitment of WRN to the sites of DNA damage.

This project is supported by the Sassella-Stiftung and the Marie Heim-Vögtlin Stiftung. It was carried out in collaboration with Prof. Igor Stagljar of the Institute of Veterinary Biochemistry and Molecular Biology, University of Zurich.

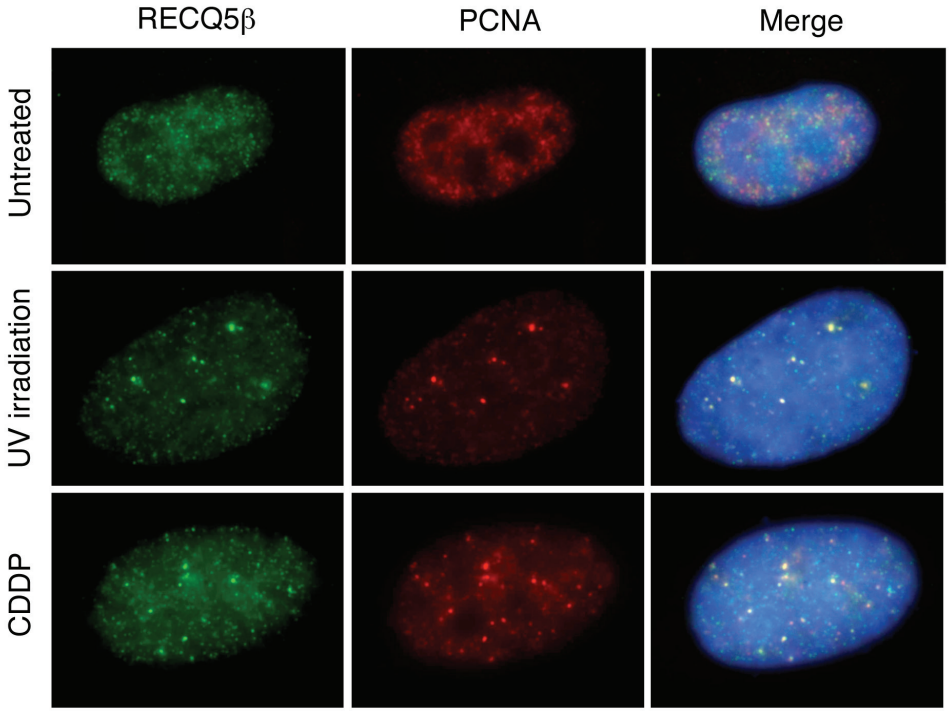

Figure 1: Co-localization of RECQ5β with the DNA replication factor PCNA at nuclear foci following exposure of HeLa cells to UVC irradiation or cis-platinum (CDDP) treatment, which can cause replication fork stalling.

Molecular mechanisms of DNA damage tolerance in human cells

Sybille Schwendener

DNA damage poses a serious threat to each proliferating cell as it can inhibit progression of replication forks. Replication-fork stalling can induce unscheduled DNA recombination events and hence lead to gross chromosomal rearrangements that can cause tumorigenesis. To avoid these detrimental consequences of DNA replication arrest, cells can switch to different DNA-damage bypass modes that permit replication across the lesion. One of these mechanisms involves a transient template switch to the undamaged sister chromatid, allowing the replicative polymerase to synthesize the sequence complementary to the blocking lesion in an error-free manner (Figure 2). It is believed that this template switching is mediated by fork regression, a DNA transaction that includes unwinding of the newly replicated arms of the fork, annealing of the nascent DNA strands and re-pairing of the parental strands to form a Holliday-junction structure. Indeed, Holliday junctions have been observed to accumulate upon replication arrest in both prokaryotic and eukaryotic cells. However, it is not clear whether the formation of these structures is promoted enzymatically or occurs spontaneously. Our aim is to identify proteins that mediate replication-fork regression in human cells.

This project is funded by Bonizzi-Theler Stiftung. It was carried out in collaboration with Dr. Leonard Wu and Prof. Ian D. Hickson, University of Oxford.

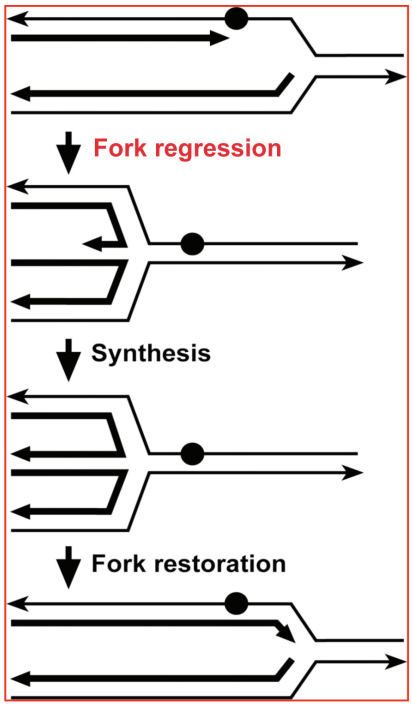

Figure 2. DNA damage bypass by template switching

Mechanism of DNA translocation and unwinding by RecQ helicases

Patrick Garcia

The knowledge of the mode of action of RecQ DNA helicases is required for better understanding of the DNA transactions mediated by these anti-recombinases in vivo. We have addressed the mechanism of DNA unwinding by the human RecQ helicase BLM, which is known to suppress crossing-over during HR by acting in conjunction with Topoisomarase III to decatenate recombination intermediates containing double Holliday junctions. Most of the known 3'-5' DNA helicases are members of superfamilies 1 and 2 (SF1 and SF2). These helicases have similar sets of conserved motifs, but appear to differ in the mode of interaction with the DNA template. The SF1 helicases interact with ssDNA using mainly hydrophobic interactions with the DNA bases, whereas the SF2 helicases such as BLM seem to interact with the DNA via non-specific electrostatic interactions with the phosphodiester backbone. We found that restricting the rotational flexibility of the phosphodiester backbone in the translocating DNA strand by introducing a tandem of vinylphosphonate internucleotide linkages significantly inhibited the helicase activity of BLM. However, this inhibitory effect was not as drastic as that observed for the SF1 helicase PcrA, which is known to translocate along ssDNA solely via a base-flipping mechanism, and it was completely relieved by the DNA single-strand binding protein RPA. Moreover, we found that BLM did not induce DNA supercoiling when anchored to covalently closed circular DNA via fusion to a site-specific DNA binding domain, excluding the posibility that it is capable of translocating along dsDNA. Based on these and previously published data, we concluded that BLM translocates along ssDNA to mediate DNA unwinding, utilizing a combination of base-flipping and phosphodiester backbone interactions.

This project is funded by the Swiss National Science Foundation. It was carried out in collaboration with Dr. Panos Soultanas of the University of Nottingham.

Eukaryotic Mismatch Repair

Prof. Dr. Josef Jiricny

Anne Anstett
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Franziska Fischer
Nina Mojas
Lovorka Stojic

Rea Andermatt
Emilija Veljkovic
Torsten Kleffmann

Our group is 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 are trying to understand how MMR malfunction leads to malignant transformation. We are also studying the role of MMR proteins in other pathways of DNA metabolism.

Reconstitution of the human MMR system

Katja Bärenfaller, Elda Cannavo, Bertran Gerrits

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 being expressed in the laboratory of our collaborator, Ulrich H¸bscher. Although the minimal MMR system was recently reconstituted in the laboratories of Paul Modrich and Guo-Min Li, there are a number of mechanistic issues that remain to be resolved. Moreover, we need to have the recombinant system working in the laboratory in order to achieve our other objectives.

One of these is the analysis of the protein/protein interactions during the repair process. We have some very interesting mutants of the MMR proteins and we plan to deploy these in the molecular dissection of the MMR process. We are also attempting to identify the redundant components of the MMR system, such as exonucleases.

The minimal reconstituted MMR system does not display the same substrate specificity as that observed in cell-free systems. This implies that some non-essential components are still missing. Using isotope-coded affinity tagging (ICAT) technology, we have been able to identify a large number of peptides that bound preferentially to the mismatch-containing substrate in MMR-proficient extracts. We are currently in the process of verifying the participation of these polypeptides in the MMR process by biochemical means.

We also set out to complement the ICAT experiments with tandem affinity purification (TAP-tagging). In the first experiments, hMLH1 or hPMS2 fused to an affinity tag (TAP) were stably expressed in 293T cells, which lack the wild type hMLH1. 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.unizh.ch), we have now identified also a series of other possible partners of these polypeptides, which are currently being evaluated.

Repair of modified nucleotides

Petr Cejka, Franziska Fischer, Nina Mojas, Lovorka Stojic

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, such that MMR-deficient cells are up to 100-fold more resistant to killing by methylating agents of the SN1 type than their MMR-proficient counterparts. MMR-deficient cells have also been reported to be resistant to cisplatin, doxorubicin, etoposide and ionizing radiation, but our most recent evidence shows that only the resistance to methylating agents and cisplatin is MMR-dependent. In order to understand the molecular basis of the resistance phenomenon, we need to test whether DNA substrates carrying defined base modifications are addressed by the MMR system *in vitro*. This has not been tested to date, as substrates carrying the desired modifications in the template strand could not be prepared to date. We have recently been able to devise a method of preparing such substrates, using a combination of primer extension reactions on single-stranded substrates and "nickase" – an enzyme capable of incising specifically only a single DNA strand of its recognition sequence. We succeeded in incorporating 6-O-methylguanine into our substrates, and were able to show that this substrate is addressed by the MMR system both when paired with cytosine and when mispaired with thymine.

MMR in DNA-damage induced signal transduction
Franziska Fischer, Nina Mojas, Lovorka Stojic

MMR has been shown to be required for the induction of cell death following treatment of cells with methylating agents. ating agents. The mechanism of this process has been postulated to involve "futile cycles of repair", in which the MMR system is repeatedly processing the newly-synthesised strand, because it is unable to remove the damaged base from the template strand. We have been searching for experimental evidence that would substantiate this hypothesis. To date, we have no indication after the second S-phase following treatment, which is unique. We were also able to implicate the ATR kinase in the process. This suggested to us that the lesions triggering the cell cycle arrest were generated during DNA replication and we are currently putting a great deal of effort into the identification of the lesions that trigger the process. What we were able to show is that activation of the arrest was accompanied by the formation of long stretches of single-stranded DNA, as demonstrated in vivo by the formation of foci of the single strand binding protein RPA. These foci co-localised with those of a polypeptide that is exclusively modified by ATR. Foci of phosphorylated histone H2AX, which are synonymous with double strand breaks, appeared later and did not co-localise efficiently with those of RPA, which implied either that RPA was being displaced from the sites of double strand breaks, or that these breaks did not arise at arrested replication forks.

Interaction of MMR with other pathways of DNA metabolism

Petr Cejka, Nina Mojas

MMR has been shown to be required for the induction of cell death following treatment of cells with methylthat this iterative repair process is operational, at last
not
in the in vitro system that we are using.
There is an accumulating body of evidence that points to a role of MMR proteins in DNA damage signalling. Using the "gold standard" 293T La cell line developed in our laboratory, in which the expression of hMLH1 can be turned on and off at will by doxycycline, we were able to show that the signalling cascade is activated only

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 this agent, and arrested already in the first S/G2 phase post treatment. This suggested that recombination rescued the cells after the first replication.
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 by two orders of magnitude. This exciting finding enabled us to screen the complete library of viable yeast deletion mutants (made available to us by Matthias Peter) for genes participating in MMR- and recombination-mediated processing of methylation damage.
In order to gain further insights into the cross-talk between MMR and other pathways of DNA repair and metabolism, we constructed isogenic yeast strains deficient in nucleotide excision repair, lesion bypass and postreplication repair, together with double and triple mutants (where possible). The phenotypes of these strains in response to treatment with cisplatin or other reagents should help us identify, through epistatic analysis, the pathway(s) that interact (or interfere) with one another.

Visualisation of MMR in vivo and in vitro

Rea Andermatt, Nina Mojas

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.
More recently, we were able to isolate stable clones expressing eGFP-hMLH1 in a hMLH1-deficient cell line. Interestingly, although both clones express comparable levels of the hMLH1/hPMS2 heterodimer, one arrests upon DNA damage and the other doesn't. We are currently characterising these cell clones in greater detail. Some of the work is being carried out in collaboration with Jiri Lukas.

Alternative MMR

Elda Cannavo

Although it is known that MMR involves principally the heterodimeric factors hMSH2/hMSH6 and hMLH1/hPMS2, there is evidence that other heterodimers, such as hMSH2/hMSH3, hMLH1/hPMS1 and hMLH1/hMLH3 exist in human cells in vivo. We have previously examined the hMLH1/hPMS1 heterodimer and failed to find any evidence of its involvement in the MMR process in vitro.
The MSH2/MSH3 (hMutSb) heterodimer does function in MMR, in the repair of IDLs, and genetic work in yeast has shown that this factor may interact not only with MLH1/PMS1 (in yeast, PMS1 is the functional homologue of hPMS2), but also with MLH1/MLH3. We could show that the hMLH1/hMLH3 (hMutLg) heterodimer does indeed participate in MMR in vitro, although its activity is very low compared to hMutLa. Surprisingly, hMutLg did not appear to be involved in the processing of IDLs together with hMutSb, but rather in the repair of base-base mismatches and single nucleotide IDLs, together with hMutSa. This was supported by the finding that Mlh3 knock-out mice have a small but reproducible defect in the correction of mononucleotide repeats.

The role of MSH1 protein of S. cerevisiae in DNA repair

Torsten Kleffmann, in collaboration with Elaine Sia, University of Rochester

As its name implies, MutS homolog 1 (MSH1) was the first characterized eukaryotic homolog of the mismatch binding bacterial protein MutS. Although it was discovered in 1992 and shown to be a mitochondrial protein and to bind to mismatches in vitro, its role in DNA repair has not been elucidated. Given that the known MutS proteins have no endo- or exonucleolytic activity, they cannot repair DNA on their own. In all organisms that possess a MMR system, MutS proteins form heterodimers that act as mismatch recognition factors and that mediate repair by interacting with a multitude of other proteins. Yeast strains deficient in MSH1 have a severe phenotype, but it could not be shown how the protein functions, or which proteins it interacts with.
We have been able to obtain a msh1 S. cerevisiae strain from Elaine Sia, which expresses a TAP-tagged MSH1. We are currently attempting to isolate MSH1-containing multi-protein complexes and to analyse them by mass spectrometry at the FGCZ.

Transcritomic analysis of lung cancer

Emilija Veljkovic, Anne Anstett

Lung cancer is a very complex and heterogeneous malignancy. The primary cause of lung cancer in the civilised world in cigarette smoke, which contains a large number of mutagenic and clastogenic substances. Although the principal cancer-causative ingredients of cigarette smoke have been identified, the mechanism of transformation of lung epithelial cells to malignant tumour cells is unknown. We were approached by Philip Morris with the request to analyse the cigarette smoke-induced transformation process by transcriptomic and genomic approaches, with a specific focus on gene inactivation by cytosine methylation. We are deploying two strategies. In one approach, we are treating lung epithelial cells with chronic or acute doses of cigarette smoke condensate and analyzing their tumorigenic potential as well as the treatment-associated changes in their transcritpome. In the other series of experiments, we are treating lung cancer cells with the DNA demethylating agents 5-deoxyazacytidine and trichostatin-A. The transcriptome of the demethylated cells is then compared with that of the untreated controls, as well as with the transcriptome of the cigarette smoke condensate-treated cells.

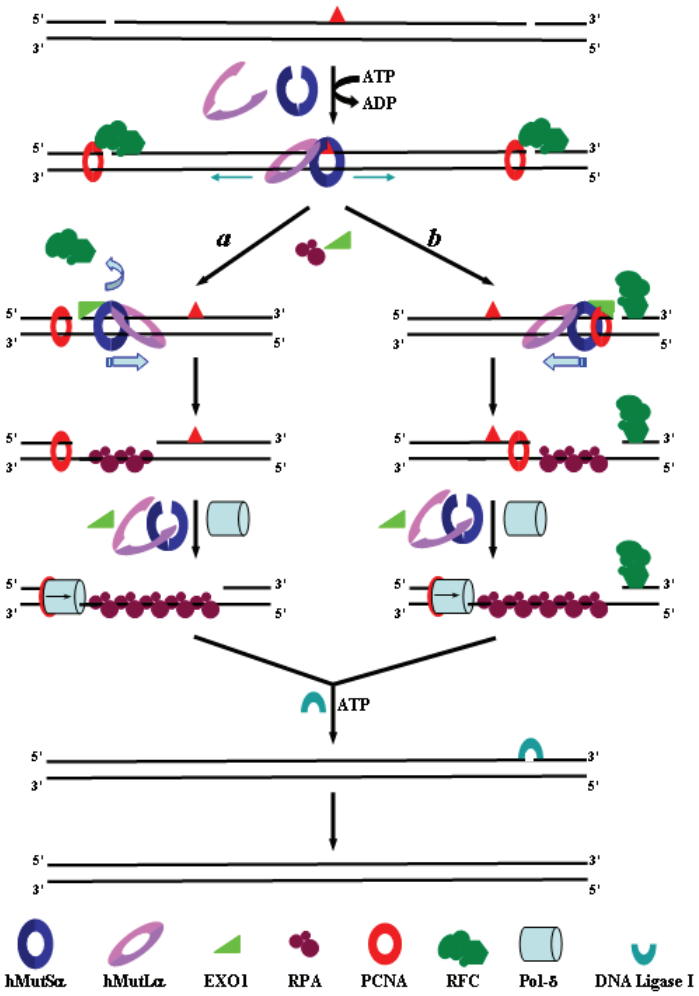


Figure 1: Reconstituted human MMR system. The mismatch-bound hMutSα (or β) recruits hMutLα. The ternary complex undergoes an ATP-driven conformational switch, which releases the sliding clamp from the mismatch site. (a) Clamps diffusing upstream that encounter a nick or a gap will load and activate EXO1, which will commence degradation of the strand in a 5'→3' direction. The single-stranded gap is stabilised by RPA. When the mismatch is removed, EXO1 activity is no longer stimulated. Polymerase-δ can load at the 3' terminus of the original nick or gap, which may carry a bound PCNA molecule. The complex fills the gap and DNA ligase I seals the remaining nick to complete the repair process. (b) Clamps migrating downstream that encounter a PCNA molecule bound at the 3' terminus of a nick or a gap recruit and activate EXO1, which degrades the strand in a 3'→5' direction. Once the mismatch is removed and the EXO1 activity is no longer stimulated, RFC loads PCNA at the 3' terminus of the newly-generated gap, which is filled-in by polymerase-δ. DNA ligase I seals the remaining nick to complete the repair process. RFC also prevents the degradation of the downstream nick in the 5'→3' direction (not shown).

Colon cancer

Dr. Giancarlo Marra

Massimiliano di Pietro / PhD Student
Mirco Menigatti
Jacob Sabates-Bellver
Kaspar Truninger
Ritva Haider / Technician

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. However, 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), widespread genetic instability involving primarily mono- and di-nucleotide repeats. In comparison to 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 cisplatin, and appear to be less responsive to standard fluorouracil-based adjuvant chemotherapy. Alternative forms of treatment are therefore mandatory (reviewed in refs. 1,2,17).
Our group is engaged on projects aimed at the identification of molecular markers of colon cancer formation and progression, with particular emphasis on those specific to MMR(-) tumors. To this end, we deploy several techniques of molecular biology, biochemistry and cell biology, together with high-throughput screening procedures such as microarray analysis of the transcriptomes of the tumors. The basic research performed in this laboratory is strictly connected to the clinic, in particular to the work of pathologists, gastroenterologists and oncologists of the University of Zurich and other clinical centers in this town.

Giancarlo Marra

Transcriptomic analysis of colonic adenomas and carcinomas

Elisa Cattaneo, Massimiliano di Pietro, Jacob Sabates-Bellver

This project focuses on the comparative analysis of the transcriptomes of adenomatous and cancerous lesions of the colorectum and those of the normal mucosa of two intestinal segments, the ileum and the colon, which are characterized by low and high rates of cancer incidence respectively. The tissue was collected at the Triemli Hospital Zurich, in collaboration with Dr. F. Bannwart and Dr. A. Schnider of the Institute of Pathology and Surgery, respectively, and at the Belcolle Hospital in Viterbo, Italy, in collaboration with the head of its Gastroenterology Department, Prof. M. Anti. The tissue was analyzed using Affymetrix U133Plus2.0 microarrays, which include in situ synthesized oligonucleotides (54,675 probes) covering the entire human transcriptome. Hierarchical clustering analyses of the gene expression data effectively segregated the four tissue categories and led to the identification of hundreds of genes, the differential expression of which in these tissues is expected to shed light on the molecular processes involved in colon carcinogenesis (Figure 1). Thus far, this study has yielded the molecular signature of a particular subgroup of colon cancers caused by defective DNA mismatch repair (15). We are currently analyzing the global differences in gene expression between colorectal tumors and normal colonic mucosa.
More recently, we have focused on a group of about 200 genes, the function of which is known or suspected to be related to Wnt signalling. This is a collaborative work with Prof. H. Clevers of the University of Utrecht. The recently-acquired laser tissue microdissector (Molecular Machines & Industries) will allow us to identify changes of transcript levels in specific regions of tumors and normal tissue.
The transcriptomic approach has also been used to characterize the changes of transcript levels in MMR(-) and MMR(+) cells upon treatment with methylating agents (7). This study was conducted in conjunction with other studies aimed at the biochemical characterization of the MMR-dependent response to DNA damage by methylating agents (refs. 3,4,8-11).

Financial support: Swiss National Science Foundation, Zurich Cancer League.

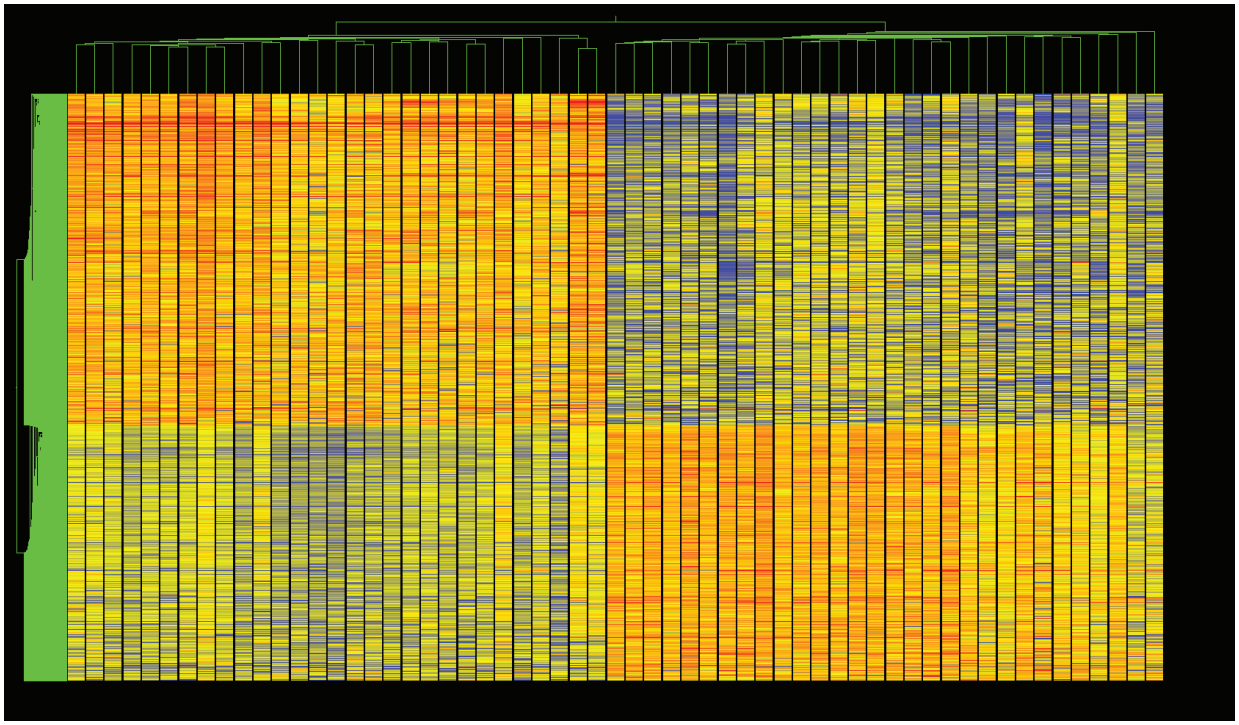


Figure 1

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

Mirco Menigatti, Kaspar Truninger (currently at the Kantonspital Aaarau) Technical assistance: Ritva Haider

We set out to establish the true frequency of colorectal cancers with defective MMR. To this end, we analyzed 1048 unselected, consecutive colorectal cancers. We then focused on PMS2-deficient cancers, as aberrant expression of this MMR protein has to date been documented only in extremely rare cases, in spite of the essential role of this polypeptide in the MMR process. Expression of the MMR proteins MSH2, MSH6, MLH1 and PMS2 was studied by immunohistochemistry. Where absence of MMR proteins was detected, microsatellite instability and cytosine methylation status of the respective MMR gene promoter were determined. DNA of patients presenting with PMS2-deficient CRCs was searched for the presence of germline and somatic alterations in the PMS2 gene.
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. As anticipated, lack of MLH1 expression was mostly associated with somatic silencing of the MLH1 promoter by cytosine methylation. PMS2-deficiency accompanied by MSI was found in 16 (1.5%) CRCs (Figure 2). Interestingly, although germline alterations in the PMS2 gene were detected, the families of patients harbouring these mutations had no obvious history of cancer. The IHC analysis revealed that PMS2 defects account for a considerable proportion of CRCs, which have hitherto been classified as sporadic. DNA-based mutation detection methods have failed to identify these cancers, due to the interference of numerous PMS2 pseudogenes, which are most likely also the underlying cause of the unorthodox inheritance pattern of these germline mutations (13).
Interestingly, most of the PMS2-deficient cases were identified in central Switzerland (Lucerne, Schwyz and Nidwalden cantons). A program with the objective to characterize the germline alterations of PMS2 in the patients and families already enrolled and to track PMS2 gene variants in the population of this region of Switzerland has been recently initiated. approved and partially financed by the Central Switzerland Cancer League.

This project was carried out in collaboration with the group of Dr. Karl Heinimann at the University of Basel. Collaborations with pathologists of several different hospitals was crucial to the success of this project. The study was supported by the Swiss National Science Foundation, the Swiss Cancer League, the Hanne-Liebermann Stiftung Zurich, Forschung in Gesundheitswesen Aarau and the Hartmann-Müller Stiftung Zurich.

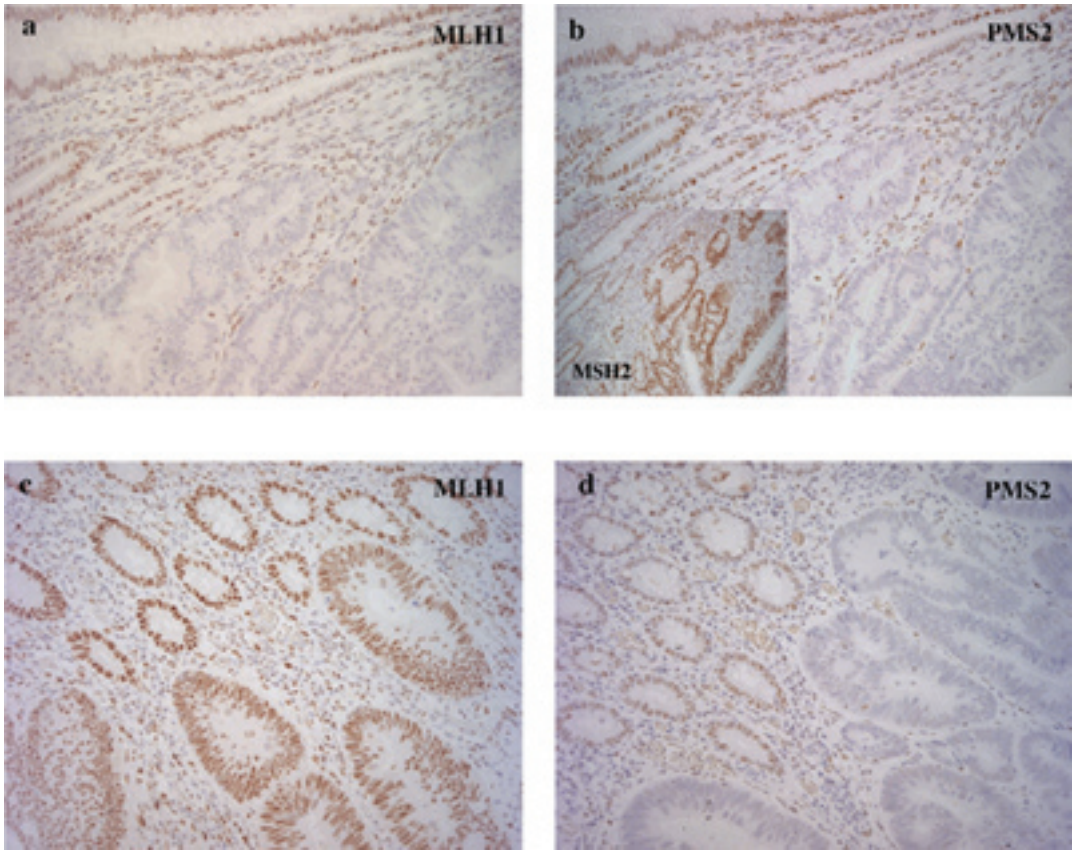


Figure 2: Immunohistochemical staining of MMR proteins in colorectal tumors.
a) MLH1 is absent from tumor tissue, but normal crypts (upper part of the picture) and proliferating stromal cells express this protein normally. b) The same tumor does not express PMS2, because this protein is unstable in the absence of MLH1. However, other MMR proteins are expressed normally, as shown for MSH2 in the inset. c) The dysplastic crypts on the right side of this tumor express MLH1 at levels similar to the normal crypts on the left, however, d) the dysplastic crypts are deficient in PMS2.

Thymine DNA glycosylase and strand break repair

Dr. Primo Schär

Dr. Christophe Kunz
Marc Bentele / PhD Student
Sanja Kais / PhD Student
Yusuke Saito/ PhD Studentr

Reactive agents of endogenous and environmental origin pose a constant threat to the integrity of our genomic material, the DNA. DNA damage, if it goes unrepaired, destabilizes genomes and, thus, increases the risk of cancer. We explore biological processes that enforce genome stability at the level of DNA damage response and repair. The objective of our work is to provide a clear understanding of both, the molecular mechanisms involved in the repair of DNA base damage and broken DNA backbones, and the biological consequences of their dysfunction.

Thymine DNA-Glycosylase- Antimutator and Tumour Suppressor?

Marc Bentele, Christoph Kunz

Restoration of G:C pairs from G:T/U mismatches is accomplished by a short patch base excision repair process, in which thymine DNA-glycosylase (TDG) appears to play a central role. We generated Schizosaccharomyces pombe strains mutated in the fission yeast ortholog of TDG, thp1, and the functionally-related uracil DNA glycosylase (ung), to study the role of Thp1p in the antimutagenic DNA repair process. We could show that inactivation of thp1 in the background of a uracil DNA glycosylase deficiency (udg-) causes a specific C→T mutator phenotype. Moreover, we found that inactivation of uracil DNA glycosylase sensitizes cells to the therapeutic anti-tumour drug 5-fluorouracil (5-FU) and that this cytotoxicity is largely accounted for by the action of Thp1p. Another important observation was that Tdg appears to induce a substantial fraction of spontaneous mitotic recombination events between duplicated marker genes and may therefore significantly contribute to gross genomic instability. Together, these observations provided the first evidence for the involvement of Tdg, in conjunction with Ung, in the processing of damaged bases in living cells. Current work with the yeast model is directed towards disentangling the complex biology of the repair of DNA base damage. Having observed that the repair of uracil in DNA contributes significantly to spontaneous mitotic recombination, our objective is to understand in detail the relationship between the occurrence and the repair (or misrepair) of DNA base damage and spontaneous gross genomic instability. An important recent finding suggests that coordination of the Thp1p- and Ung1p-controlled pathways is achieved through S-phase specific DNA damage signalling. We found that Cds1p as well as Chk1p affect the choice of the pathway and thus the qualitative outcome of the respective repair processes.

Biological roles of TDG mediated base excision repair in mammalian cells

Christophe Kunz, Yusuke Saito

We started to establish mouse knockout and cell culture models to investigate the function of TDG in mammalian cells. Embryos lacking Tdg died at 10.5-11.0 days post-coitum for reasons that are currently unknown. This dramatic phenotype is unusual for a DNA glycosylase defect and may therefore reflect the function of TDG in gene regulatory processes that occur during mammalian development, perhaps in keeping CpG islands of active genes free of cytosine methylation. We generated homozygous Tdg-/- cell lines from knockout embryos and from heterozygous ES cells, respectively. The phenotype of these cell lines is being investigated. Consistent with a role of Tdg in gene regulation, preliminary analyses of genome wide-gene expression patterns revealed dramatic differentiation-induced differences between Tdg knockout and wild-type cells. We are currently in the process of generating a conditional Tdg knockout, taking advantage of the Cre-Lox system.

The role of posttranslational modifications in DNA base excision repair

Roland Steinacher

We showed that TDG is a target for covalent modification by the SUMO proteins. We then found that SUMO-conjugation dramatically reduces the DNA substrate and AP site binding affinity of TDG, and that this is associated with a significant increase in enzymatic turnover. These observations implicated SUMOylation in the controlled dissociation of TDG from the AP site. To better understand the structural concept underlying the product inhibition of TDG and the molecular mechanism of SUMOylation-induced turnover, we performed structure-

function studies. These revealed that TDG undergoes a conformational change upon DNA binding and identified its non-conserved N-terminus as a flexible extension that cooperates functionally with the catalytic domain to mediate tight DNA binding. By analyzing the turnover kinetics of different TDG variants, SUMO-conjugated and unmodified, we were able to show that SUMOylation in the C-terminus modulates the conformation of the N-terminus without affecting the functionality of the glycosylase active site. We can thus postulate a mechanism for coordinated release of TDG from the product AP-site, whereby SUMOylation effects opening of the DNA binding clamp formed by the N-terminus and the core domain of the glycosylase.

Regulatory mechanisms of DNA double-strand break repair in yeast

Sanja Kais

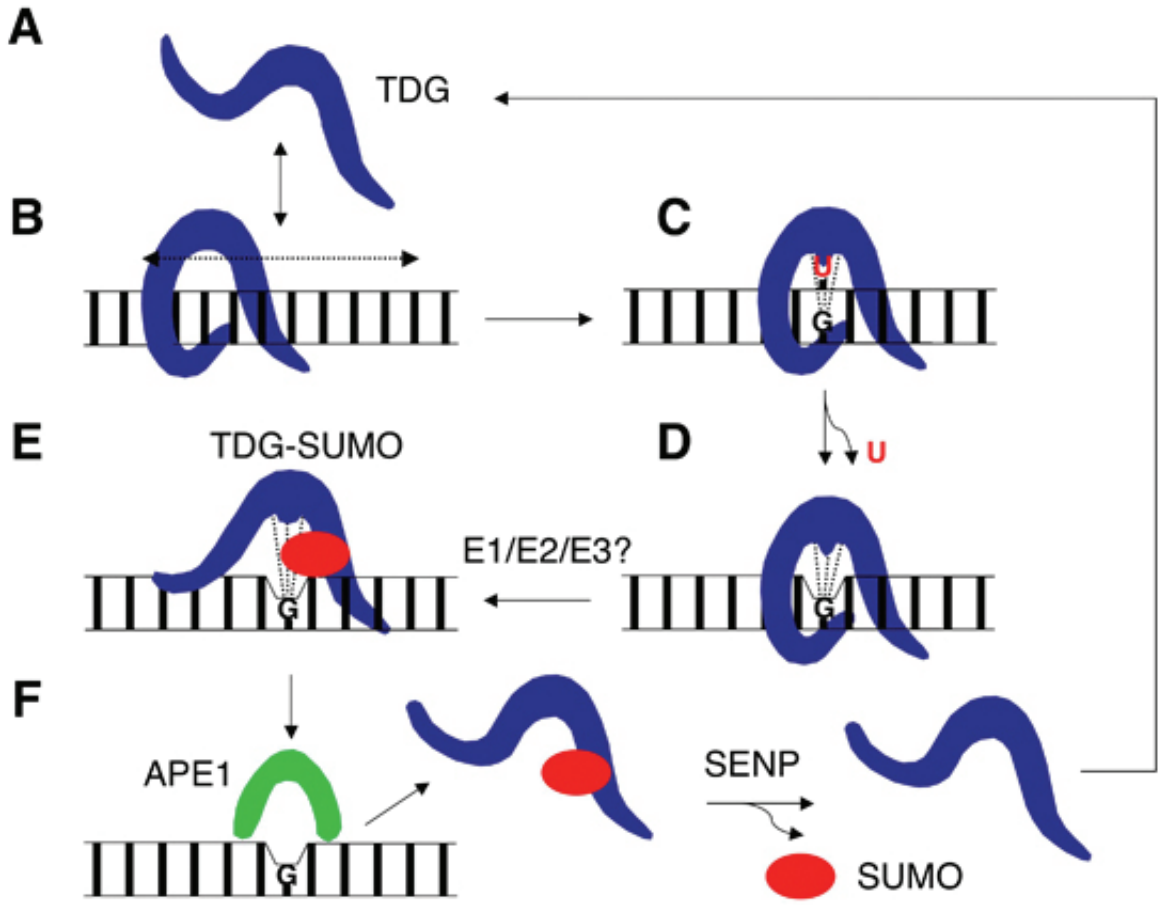
Genomic instability is a hallmark of the majority of human tumours. Chromosomal rearrangements represent the prevalent form of instability, suggesting an underlying malfunction of the process of DNA double-strand break repair. Double-strand breaks (DSBs) represent a severe form of DNA damage induced by ionizing radiation or the interaction of DNA with reactive chemicals of endogenous or environmental origin. More frequently, however, they occur as a consequence of normal DNA metabolism involved in cell proliferation (DNA replication) and differentiation (V(D)J recombination, meiosis). Eukaryotic cells make use of two distinct recombination pathways to repair DSBs: homologous recombination (HR) and non-homologous-end-joining (NHEJ). Inactivation of either of these pathways gives rise to chromosomal aberrations that frequently result from irregular repair of DSBs by the inappropriate pathway. Thus, an accurate coordination of DSB repair in cells is of critical importance for genome stability and suppression of tumour development.

We have been studying NHEJ in the yeast model with a particular interest in identifying and characterizing regulatory components of this pathway. In this context, we isolated two previously unidentified yeast proteins, Nej1p and Nej2p. In collaboration with the group of James Haber at Brandeis University, we could show that Nej1p is an essential component of the NHEJ system. Current work addresses the molecular function of Nej1p in NHEJ.

The second newly-discovered Lif1p interacting protein, Nej2p, is a conserved G-patch containing protein with a postulated RNA binding and processing function. Like the yeast Nej2p, the human ortholog interacts specifically with XRCC4. We initiated projects to investigate genetically, cytogenetically and biochemically the molecular details of a possible role of yeast and human Nej2p in NHEJ. We found that the Nej2p/NEJ2 interaction with Lif1p/XRCC4 occupies the Dnl4p/LIG4 binding site, preventing the formation of an active ligase complex. Consistently, overexpression of Nej2p negatively affects NHEJ in a plasmid re-ligation as well as in a chromosomal DSBR assay. However, in contrast to other factors involved in NHEJ, NEJ2 is essential for survival in yeast, indicating multifunctionality. Both yeast and human NEJ2 also interact with another G-patch protein, PinX1, which localizes to the nucleolus and to telomeres and which has been implicated in the regulation of telomerase activity. These findings suggest a possible novel mechanism of regulating NHEJ by Nej2p/NEJ dependent sequestering Lif1p/XRCC4 in an inactive complex.

Structural Maintenance of Chromosomes (SMC) proteins act in different DNA dynamic processes. Involvement of the SMC1/SMC3 heterodimer in DNA double-strand break repair (DSBR) emerged from biochemical dissection of a mammalian protein complex with DNA strand exchange activity, from genetic studies of meiotic recombination in yeast and from studies of an ATM-dependent DNA damage response of SMC1. In collaboration with Rolf Jessberger at the University of mmmm, we studied functional interactions between SMC1 or SMC2 and the homologous recombination (HR) and non-homologous end-joining (NHEJ) pathways for DSB repair in Saccharomyces cerevisiae. Our data indicate that Smc1p may function in the coordination of double strand break repair processes.

The above projects have been funded through the Swiss National Science Foundation (SNF), Association for International Cancer Research, Sassella Stiftung, Bonizzi-Theler Stiftung, UBS AG and the Julius Müller Stiftung.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Mechanisms of intra- and inter-strand cross-links

Dr. Orlando Schärer

Dr. Rolf Buff
Ludovic Gillet / PhD Student
Jerome Gualbert / PhD Student
Barbara Orelli / PhD Student
Lidija Staresincic / PhD Student
Muriel Träxler / Technician

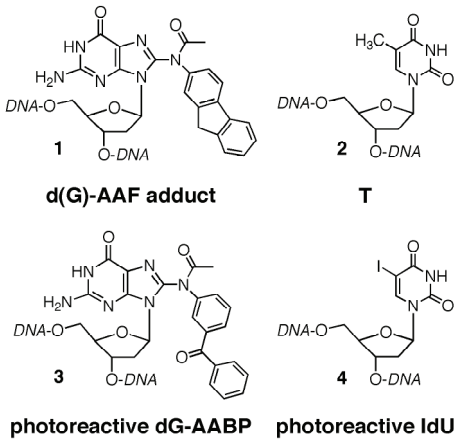
Our research combines organic chemistry, biochemistry, and molecular and cellular biology to investigate the mechanisms of DNA repair in mammals and the relationship of these processes to anti-tumor and gene therapy.

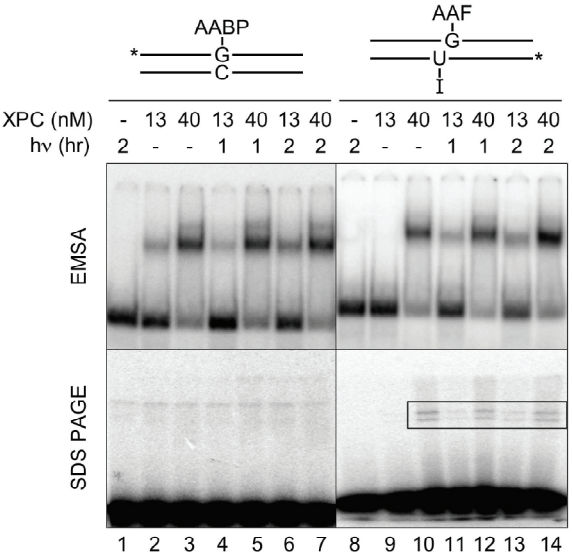
Molecular mechanisms of nucleotide excision repair

Rolf Buff, Ludovic Gillet, Jerome Gualbert , Barbara Orelli, Lidija Staresincic, Muriel Träxler

Nucleotide excision repair (NER) is the main pathway by which bulky lesions are repaired. Defects in NER are associated with the hereditary syndrome Xeroderma pigmentosum, which is characterized by extreme UV sensitivity and a more than 2000-fold increase in cancer incidence. NER is mediated by the coordinated action of over 30 proteins and includes the following steps: damage recognition, helix opening and open intermediate stabilization, dual incision flanking the lesion, removal of the damaged oligonucleotide, gap-filling by replication proteins and ligation of the nick. As the NER reaction has been reconstituted using purified proteins, it represents an ideal system to study molecular recognition and catalysis by a multi-protein complex. Our studies on NER are focused on elucidating how proteins involved in NER cooperate in the recognition and excision of the damaged sites.

We have designed and synthesized photoreactive and fluorescent analogs of an acetyl aminofluorene adduct of dG, an efficient NER substrate, to investigate the mechanisms of damage recognition in NER. Our photocross-linking studies have revealed that the initial damage recognition protein XPC/HR23B specifically interacts with the non-damaged strand of DNA opposite the lesion, rather than the lesion itself (Figure 1). These findings provide a mechanistic rationale for earlier observtions that have proposed that efficient NER substrates induce significant helical distortion in addition to containing a chemical modification. Thus HR23B/XPC apparently interacts with the distorted non-damaged strand opposing the lesion rather than the lesion itself. A second protein is then required to verify the presence of the lesion. Future studies include the identification of this damage verification protein using photoreactive NER substrates. Furthermore, we will use fluorescent NER substrates together with fluorescently labeled NER proteins to study how various NER factors interact with substrates and each other in real time in vitro and in vivo.





Orlando Schärer

A second aspect of our work on NER has focused on the two NER endonucleases ERCC1/XPF and XPG. We have identified and characterized the active site of ERCC1/XPF, a member of a new nuclease family. Our work on XPG has revealed that this protein has distinct requirements for binding and cleaving substrates, a property that we believe plays an important role in regulation of the NER pathway. We have separated binding and catalysis by ERCC1/XPF and XPG by protein or substrate modification, a property that we are now exploiting for the characterization of reaction intermediates in NER. Another project tests the hypothesis that the transition from the excision to the repair synthesis steps in NER is highly regulated and involves the sequential order of incision of the damaged oligonucleotide by the two nucleases.

DNA interstrand crosslink repair in mammals

Todor Angelov, Jawad Alzeer , Milica Enoiu, Angelo Guainazzi, Vinh Ho

DNA interstrand crosslinks (ICLs) are the physiologically most relevant adducts formed by a number of anti tumor agents in clinical use, yet it remains poorly understood how they are repaired in mammalian cells. One of the problems in studying ICL repair has been the limited availability of defined ICLs for biochemical and cell biological studies. We have found a solution to this problem by incorporating ICL precursors on complementary strands of DNA using solid-phase synthesis and using a specific reductive amination coupling reaction to furnish the ICL (Figure 2). This methods has allowed us the prepare ICLs in high yield and purity. We are employing these crosslinks in a reporter assay that we have designed to investigate pathways of ICL repair in living human cells. This assay will be used to determine which genes are involved in the major, replication-dependent and minor, replication-independent ICL repair pathways. These studies will be used to generate hypotheses for ICL repair pathways, which will then be investigated at the biochemical level.

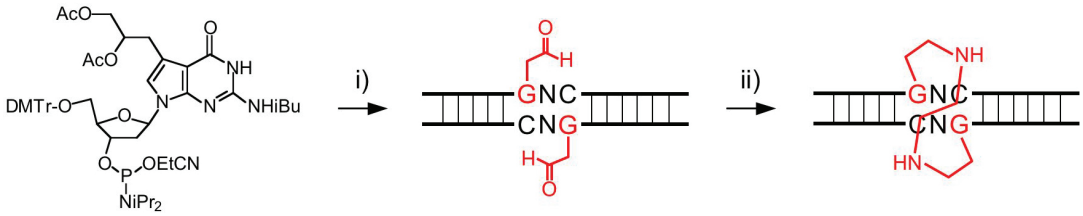


Figure 2: Syntheis of Nitrogen mustard ICL mimic using post-synthetically modifiable ICL precursors. i) A phosphoramidite containing a protected 2,3-propane diol side chain at the 7 position of deazaguanosine is incorporated into complementary strands of DNA. Under basic deprotection conditions, the corresponding diol is formed and oxidized to an ethanal with NaIO4. iv) Reductive amination with diaminoethane in the presence of NaBH3CN yields an ICL in the major groove of DNA.

A DNA repair-based approach to gene therapy and targeted mutagenesis

Jawad Alzeer

Our third objective is to develop strategies to induce single base pair changes in genomic DNA in living cells by exploiting the DNA repair machinery. The ability to modify single base pairs in genes in living cells and organisms would not only be important for functional genomic studies, but will also provide a novel strategy for gene therapy. As a first step toward this goal we have generated oligonucleotides containing a modified base capable of 1) covalently modifying a base opposite to it through a transfer of a functional group, thereby activating it as a substrate for a DNA repair pathway and 2) subsequently serve as a template for repair synthesis. We are presently optimizing this reaction and will test whether this functional group transfer reaction can indeed induce single base substitutions at specific sites of reporter genes in living cells.

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Reviews

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Editorials

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2005

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Book Chapters

Marra G & Jiricny J.
DNA Mismatch repair and colon cancer. In "Genome Instability in Cancer Development". Editor: Erich Nigg. Springer Science+Business Media B.V., Formerly Kluwer Academic Publishers B.V.

Teaching

Teaching

J. Jiricny

Basic Molecular Biology
Biochemistry I, for Medics
Biochemistry II, for Students of Biochemistry and Chemistry

J. Jiricny and G. Marra

Tutorials in Oncology and Molecular Biology

J. Jiricny and colleagues

Radiobiological Seminars
DNA Damage Repair and Cancer
Repair, Replication and Recombination Club

P. Schär

Molecular Genetics (Medical Faculty of the University of Basel)
Genome (In)stability (Phil.-Nat. Faculty of the University of Bern)

O. Schärer

Biological Chemistry I (Department of Chemistry at the ETH Zurich)

Collaborations

Collaborations

Brandeis University, Waltham, USA
Cancer Research UK, London Research Institute, UK
CRC laboratories, UK
Erasmus University, Rotterdam, The Netherlands
ETH Zurich, Switzerland
Friedrich Miescher Institute, Basel, Switzerland
Harvard Medical School, Cambridge, USA
IGBMC, Strasbourg, France
Imperial College London, UK
Institute of Veterinary Biochemistry, University of Zurich, Switzerland
Istituto Dermopatico dell'Immacolata, Rome, Italy
Johns Hopkins School of Medicine, Baltimore, USA
Cantonal Hospital Aarau, Switzerland
Massachusetts Institute of Technology, Cambridge, USA
Mount Sinai School of Medicine, New York, USA
Novartis Pharmaceuticals, Basel, Switzerland
Triemli Hospital, Zurich, Switzerland
Universität Köln, Germany
University of Basel, Switzerland
University of Dundee, Scotland, UK
University of Edinburgh, UK
University of Geneva, Switzerland
University of Michigan, USA
University of Nottingham, UK
University of Oxford, England, UK
University of Padua, Italy
University of Texas Health Science Center, San Antonio, USA
Vanderbilt University, Tennessee, USA
Waidspital, Zurich, Switzerland

Conferences

J. Jiricny organised the 1st Cancer Research Retreat Zurich, Switzerland

J. Jiricny organised the 6st Charles Rodolphe Brupbacher Symposium Zurich, Switzerland

G. Marra organised the Cancer Network Zurich meeting on colon cancer, Zurich, Switzerland

O. Schärer organized the 2nd EMBO Young Investigator Meeting: "Chemistry Meets Biology: Chemical Ap-proaches to the Study of Biology" at the EMBL Heidelberg, Germany

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

S. Ferrari

Institute of Cell Biology, University of Bern, Switzerland
International Graduate School of Chemistry, University of Muenster, Germany
Novartis Institutes for Biomedical Research, Basel, Switzerland

J. Jiricny

ISREC, Lausanne, Switzerland
ZLF, Kantonsspital Basel, Switzerland
Society of Pharmacology, Bern, Switzerland
Alumni of University of Zürich, Switzerland
Swiss Medical Genetics Society, Zürich, Switzerland
Academy of Sciences, Prague, Czech Republic
Danish Cancer Institute, Copenhagen, Denmark
Joint European/USA Congress on DNA Repair and Aging, Washington D.C., USA
IFI, Milano, Italy
Leiden University Medical Center, Leiden, The Netherlands
National Cancer Centre, Amsterdam, The Netherlands
Cancer Research UK (Clare Hall), South Mimms, UK
Wellcome/Cancer Research UK, Cambridge, UK
University of Oslo, Norway
Biennial Meeting of ETH (D-Biol), Davos,Switzerland
EMBO Meeting on Genomic Instability, Gallway, Ireland
FEBS Meeting, Warsaw, Poland
BioScience 2004, Glasgow, UK
International Skin Cancer Meeting, Zurich, Switzerland
GRC on Mutagenesis, Oxford, UK
DNA Repair Congress, Bermuda, USA
Impact of Molecular Biology on Clinical Oncology, Lausanne, Switzerland
Friedrich Miescher Institute, Basel, Switzerland
Physiological Institute, University of Zurich, CH
Department of Pathology, University of Lasaunne, CH
FEBS/Howard Hughes Biochemistry meeting, Budapest, H
InSIGHT meeting, Newcastle upon Tyne, UK
MGMT 2005, Keele University, UK
5th Benzon Symposium, Copenhagen, DK
European Society of Oncology, Zürich, CH
Institut Marie Curie, Paris, F
EMBL, Heidelberg, D
2nd US/European Meeting on DNA Repair, Erice, I

P. Janscak

1st Swiss Meeting on Genome Stability – DNA Dynamics and Epigenetics, Üetendorf, Switzerland
Institute of Cell Biology, University of Bern, Switzerland

G. Marra

Institute of Cell Biology, University of Bern, Switzerland
7th Meeting of the German DNA Repair Network, Gottingen,Germany
Meeting of the Italian Society of Gastroenterology, Turin, Italy
University of Padova, Italy
Italian Society of Gastroenterology, Bari, Italy

P. Schär

Institute of Cell Biology, Bern, Switzerland
European Regional Fission Yeast Meeting, ISREC, Lausanne, Switzerland
FISV Congress, Rimini, Italy
Institute of Cell Biology, Bern, Switzerland
ISRE, Lausanne, Switzerland
Oncolunch, Kliniken Basel, Switzerland
Babraham Institute, Cambridge, UK
ESTRO 23, Amsterdam, The Netherlands

O. Schärer

Institute of Cell Biology, University of Bern, Switzerland
Dermatologische Klinik, Universitätsspital Zürich, Switzerland
Mammalian DNA repair Gordon Research Conference, Ventura, CA, USA
ISREC, Lausanne, Switzerland
Brupbacher Symposium, Zürich, Switzerland
Universita' degli Studi di Milano, Italy
1st Swiss Meeting on Genome Stability – DNA Dynamics and Epigenetics, Üetendorf, Switzerland
EPFL, Lausanne, Switzerland
Dana Farber Cancer Center, Boston, MA, USA
Dept. of Pharmacology, SUNY Stony Brook, NY, USA
2nd EMBO Young Investigator Symposium, Heidelberg, Germany
8th Meeting of the German DNA Repair Network, Ulm, Germany
8th Workshop of the Graduate Programme "Biochemistry of Nucleo-Protein complexes", Giessen, Germany
ASM Conference "DNA Repair and Mutagenesis", Southampton, Bermuda, USA

Seminars

The following lectures and seminars were given by visitors to the IMCR:

2003

Signalling via Raf-kinase.
Karin Mölling, Inst. of Medical Virology, Univ. of Zürich, Switzerland

The silent death of vision: mechanisms of apoptotic cell death in retinal degenerations.
Charlotte E. Remé, Inst. of Ophthalmology, Univ. of Zürich, Switzerland

Non-coding RNAs can silence imprinted genes
Denise P. Barlow, ÖAW Inst. of Molecular Biology, Salzburg, Austria

Molecular mechanisms of endothelial dysfunction
Thomas F. Lüscher, Dept. of Internal Medicine, Univ. of Zürich, Switzerland

About oxygen sensing, HIF-1 and erythropoietin
Max Gassmann, Veterinary Physiology, Univ. of Zürich, Switzerland

Control of the cell cycle by ubiquitin-dependent degradation
Peter Matthias, Inst. of Biochemistry, ETH Zürich, Switzerland

The mammalian circadian timing system: from gene expression to behaviour and disease
Ueli Schibler, Dept. of Molecular Biology, Univ. of Geneva, Switzerland

Function of Dicer in RNA interference and microRNA biogenesis in mammalian cells
Witold Filipowicz, Friedrich Miescher Institute, Basel, Switzerland

Cell cycle checkpoints: mechanisms and cancer-predisposition defects
Jiri Bartek, Dept. of Cell Cycle and Cancer, Inst. of Cancer Biology, Copenhagen, Denmark

Signal transduction during Caenorhabditis elegans vulva development
Alex Hajnal, Institute of Zoology, Univ. of Zürich, Switzerland

Role of ShcA isoforms in signal transduction: analysis using siRNA
Yoshikuni Nagamine, Friedrich Miescher Institute, Basel, Switzerland

Interstrand crosslink repair in eukaryotes
Peter J. McHugh, Weatherall Inst. of Molecular Medicine, Univ. of Oxford, UK

Double-strand break repair, cancer susceptibility and p53
Lisa Wiesmüller, Inst. of Gynecological Oncology, Univ. of Ulm, Germany

Identification of protein-protein interaction sites in the DNA mismatch repair system
Peter Friedhoff, Inst. of Biochemistry, Justus-Liebig Univ. Giessen, Germany

Control of genome stability by ubiquitin and SUMO conjugation
Helle D. Ulrich, Max Planck Institute for Terrestrial Microbiology, Marburg, Germany

Cellular immune responses in HIV-1 infection
Annette Oxenius, Institute of Microbiology, ETH Zurich, Switzerland

Cancer immunotherapy: basic concepts and clinical perspectives
Alexander Knuth, Dept. of Internal Medicine, University of Zurich, Switzerland

2004

Cell cycle control and cancer
Tim Hunt, Cancer Research, Clare Hall Laboratories, South Mimms, UK

Nucleotide excision repair
Jean-Marc Egly, Institute of Genetics and Molecular Biology, Strasbourg, France

Bioinformatic and experimental studies of restriction endonucleases
Janusz M. Bujnicki, Bioinformatics Lab., International Inst. of Mol. and Cell Biology,Warsaw, Poland

Unique roles for methyl-CpG binding proteins during animal development
Richard Meehan, Dept. of Biomedical Sciences, University of Edinburgh, UK

Crossing over DNA replication and recombination intermediates
Angelos Constantinou, Department of Biochemisctry, University of Lausanne, Switzerland

A tumor suppressor in plants? Function of the Arabidopsis retinoblastoma-related protein in development and DNA replication control
Wilhelm Gruissem, Institute of Plant Sciences, ETH Zurich, Switzerland

Dynamic interactions of DNA repair and transcription factors in living cells
Wim Vermeulen, Dept. of Cell Biology and Genetics, Erasmus Univ., Rotterdam, The Netherlands

Human DNA mismatch repair in a defined system
Paul Modrich, Dept. of Biochemistry, Duke University, Durham, NC; USA

N-linked protein glycosylation: from yeast to human disease.. and back to prokaryotes
Markus Aebi, Institute of Microbiology, ETH Zurich, Switzerland

PPARs in health and disease
Walter Wahli, Centre Integratif de Genomique, University of Lausanne, Switzerland

Cell division, the centrosome cycle and chromosomal instability
Erich Nigg, Max-Planck Institute for Biochemistry, Martinsried, Germany

Translesion synthesis and the Y-family DNA polymerases
Alan Lehmann, Genome Damage and Stability Centre, University of Sessex, Brighton, UK

Of collagen and man
Cecilia Giunta, Division of Metabolism and Molecular Pediatrics, University Children's Hospital Zurich, Switzerland

The possible role of a polyomavirus in human gastrointestinal cancer
C. Richard Boland, Div. of Gastroenterology, Baylor University Medical Center, Dallas, TX, USA

DNA replication and its exploitation for cancer diagnosis
Ron Laskey, MRC Cancer Cell Unit, Hutchison MRC Research Center. Cambridge, UK

RNAi and microRNA machineries in mammalian cells
Witold Filipowicz, Friedrich Miescher Institute, Basel, Switzerland

2005

Poly(A) tales
Elmar Wahle, Institute of Biochemistry, University of Halle, D

Mechanisms of centrosome duplication in C. elegans embryos
Pierre Gönczy, ISREC, Lausanne, CH

Cancer susceptibility and the functions of the BRCA2 tumour suppressor
Ashok Venkitaraman, Hutchison/MRC Research Center, Cambridge, UK

Attenuated Familial Adenomatous Polyposis
Randall W. Burt, Huntsman Cancer Institute University of Utah, UT,USA

Genetic instability in B lymphocytes
Matthias Wabl, Univ. of California, San Francisco, USA

DNA replication in the Archaea: a robust model for Eukaryotes
Stephen D. Bell, Hutchison/MRC Research Centre, Cambridge, UK

Photoreactive DNA probes as a tool to study DNA replication and repair
Olga Lavrik, Institute of Chemical Biology and Fundamental Medicine, Novosibirsk, Russia

Familial cancer syndromes: from phenotype to genotype
Paivi Pältomaki, University of Helsinki, Finland

DNA replication and repair: versatility versus specificity
Wei Yang, NIH, Bethesda, USA

Cancer Epigenetics: breaking the DNA methylation and histone codes
Manel Esteller, Spanish National Cancer Centre, Madrid, SP

Repair at the edge: processing of 3'-end modified DNA single-strand breaks
Grigory Dianov, MRC Harwell, UK

Mechanisms of DNA damage-induced mutagenesis
Zhigang Wang, Univ. of Kentucky, Lexington, KY, USA

The enigmatic DNA polymerase epsilon - a replicative DNA polymerase with unclear function
Erik Johansson, Umea University, Sweden

DNA damage checkpoints and cancer
Thanos Halazonetis, University of Pennsylvania, USA

Scientific Advisory Board

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

Prof. Dr. Susan Gasser (director of the Friedrich Miescher Institute, Basel, Switzerland)

Prof. Dr. Walter Gehring (University of Basel, Switzerland)

Prof. Dr. Tomas Lindahl (director of the Clare Hall Laboratory of Cancer Research UK).