

# **Meeting Report DNA Repair 2008: Tenth Biennial Meeting of the German Society for Research on DNA Repair**

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## **Abstract**

The tenth meeting of the German Society for research on DNA Repair was held in Berlin in September 2008. Invited presentations by Yosef Shiloh, Stanton L. Gerson, Sacha Beneke, Patrick Concannon, Jochen Dahm-Daphi, Thilo Dörk, Friedrike Eckardt-Schupp, Bernd Epe, Ian Hickson, Ulrich Hübscher, Penny Jeggo, Malik Lutzmann, Christof Niehrs, Primo Schär and Predrag Slijepcevic together with over 80 selected oral and poster presentations generated an inspiring scientific program, which documented the impressive progress of the community and defined future challenges in the field.

## **Introduction**

The tenth meeting of the German Society for Research on DNA Repair (DGDR) was held at Campus - Rudolf Virchow of the Charité – Universitätsmedizin Berlin (local host: Institute of Human Genetics), from September 2<sup>nd</sup> to 5<sup>th</sup> 2008. The event attracted over 170 participants from 11 countries. Six plenary sessions, each with a mixture of invited lectures and proffered papers, and 4 poster sessions with 60 posters were the basis of lively discussions on current aspects of DNA repair. A keynote lecture by Yosef Shiloh and public lecture by Stanton Gerson highlighted the academic program of the meeting.

## **Keynote Lecture**

**Yosef Shiloh** (University of Tel Aviv) opened the meeting with a comprehensive overview of ATM's central role in radiation sensitivity, cancer predisposition and neurodegeneration. The emphasis of Y. Shiloh's lecture was on the newly emerging areas in which this DNA damage triggered kinase is implicated: regulation of gene expression, RNA processing and chromatin organization. In this context he presented new ATM targets such as RNF20/40 involved in histone mono-ubiquitination. Particularly with the connection to the genetic disease Ataxia telangiectasia (A-T), this lecture was an appropriate start to a meeting hosted by an institute of human genetics and was especially inspiring for the many students, who attended the meeting.

## **1: DNA Damage signalling, cell cycle checkpoints and carcinogenesis**

**Patrick Concannon** (University of Virginia) presented the first of several talks on nibrin, the product of the *NBN* gene. A combination of deletion and complementation analyses identified nuclear localization signals and a nuclear export sequence with significance for nuclear-cytoplasmic shuttling of nibrin, Mre11 localization and radiosensitivity. The experiments suggested that nibrin could have a cytoplasmic function and/or that relocation to the cytoplasm serves to down-regulate the DNA damage response after successful DNA repair. In the second part of his talk, P. Concannon addressed the role of nibrin in T cell development, a subject pertinent to the immunodeficiency of Nijmegen Breakage Syndrome (NBS) patients, who have hypomorphic mutations in the *NBN* gene. By targeting null mutations to the T precursors it could be established that TCR rearrangements were nibrin-independent and that T cell proliferation, but not viability, was nibrin-dependent.

**Thilo Dörk-Bousset** (Hannover Medical School) focused on the connection between DNA damage signalling and breast carcinogenesis. Association studies established *ATM* and several other genes of the ATM pathway as breast cancer susceptibility genes. Particularly in Eastern and Central Europe founder mutations may make a considerable contribution to the cancer burden. In some rare cases, multiple gene-gene interactions, for example heterozygous mutations in both *NBN* and *CHEK*, can modulate DNA repair efficacy and increase cancer risk. Since relative risk estimates can vary with the specific mutation, only large multi centre studies with more than 30.000 participants have the necessary power to detect weakly penetrant gene mutations.

Starting from the notion that a defect in the repair of DNA double strand breaks (DSB) alone cannot explain the extreme radiosensitivity of NBS cells, **Friederike Eckardt-Schupp** (Helmholtz Center Munich) instigated a search for additional functions. Cells depleted of nibrin by siRNA were subsequently found to be deficient in base excision repair (BER), in particular, in the polymerisation step of BER, and showed reduced PARP activity. In addition, nibrin was observed to modulate IGF-1 signalling and apoptosis after  $\gamma$ -irradiation via CD95 and PI3-K/Akt, suggesting involvement in mechanisms other than DSB repair.

Since mutations in the Poly(ADP-ribose) glycohydrolase (PARG) gene are embryonically lethal in the mouse, **Jean-Christophe Amé** (CNRS, Illkirch) generated a cellular model in which constitutive shRNA expression stably reduced PARG levels. These cells were radiosensitive and accumulated Poly(ADP-ribose) after DNA damage, displayed single-strand break repair deficiency and aberrant mitosis with supernumerary spindle poles. Thus, PARG has multiple functions including the control of mitotic progression, at least after irradiation.

The proto-oncogene, c-Fos, is required for the repair of UV-induced DNA lesions through stimulation of XPF synthesis. **Markus Christmann** (University of Mainz) demonstrated that c-Fos mediates transcriptional up-regulation of another UVC-induced gene, three prime exonuclease 1 (Trex1), and recognizes the AP-1 binding site of the *Trex1* gene. Nuclear translocation of Trex1 in replicating but not resting cells and co-immunoprecipitation of Trex1 and PolH/PCNA suggested a physiological role during replication of UV-C damaged DNA.

**Anna Melchers** (Charité – Universitätsmedizin Berlin) discussed the consequences of altering the *NBN* status *in vivo*. Mice with homozygous null mutations of *NBN* in their livers were generated using a conditional knockout model and treated with 4Gy radiation. Proteome analysis of 7000 liver proteins identified 209 differentially expressed spots revealing significant enrichment of distinct protein groups in the homozygous animals: reactive oxygen species (ROS)-related proteins, metabolic proteins, molecular chaperones. The persistent up-regulation of proteins involved in the detoxification of ROS or correction of ROS-induced lesions suggests that the primary defect, DSB repair, leads to oxidative stress and that this may significantly contribute to the pathophysiology of NBS.

**Marcus Eich** (University of Mainz) found that NBS patient cells, like those from A-T patients too, are sensitive to alkylating agents via the lesion O<sup>6</sup>-methylguanine (O6MeG). This effect is dependent on normal expression of mismatch repair proteins (MMR). Interestingly, cell death was due to necrosis rather than apoptosis. It was speculated, that nibrin and ATM cooperate in apoptosis induction after DNA alkylation. In their absence, failure to repair DSBs generated during the repair of alkylated bases would cause necrosis.

## 2: Double-Strand Break Repair I

**Penny Jeggo** (University of Sussex) focused on the impact of chromatin structure in DSB repair. Following on the observation that ATM is required for the repair of only 15 % of DSBs, she provided evidence substantiating the idea that repair of heterochromatin associated DSBs progresses more slowly and depends on ATM. This holds true for both non homologous end joining (NHEJ) in G1 and homologous recombination (HR) in G2. The requirement for ATM in DSB repair was relieved in constitutively impaired heterochromatin structures such as inactivated X-chromosomes as well as after knockdown of the heterochromatic factors KAP-1, HDAC1/2 or HP1. Furthermore, IR-induced redistribution of KAP-1 away from micrococcal nuclease-resistant heterochromatin was ATM-dependent, suggesting ATM overcomes the heterochromatin barrier to allow the repair of DSBs.

**Jochen Dahm-Daphi** (University Medical School Hamburg-Eppendorf) addressed the hierarchy of repair pathways operating on site-directed DSBs. Experiments with CHO cells carrying integrated plasmid GFP reporters with recognition sites for the endonuclease I-SceI indicated that the order of DSB repair processes is NHEJ > HR > SSA (single-strand annealing). Deficiency in any particular pathway was compensated for by use of the other pathways. More specifically, when classical NHEJ was impossible, the less accurate microhomology-dependent repair was activated. In the absence of Ku, but not XRCC4, NHEJ was dependent on PARP-activity, slow and error prone.

**Marie-Edith Chabouté** (IBMP - CNRS, Strasbourg) spoke about DSB repair in plants. Using micro RNA technology an *Arabidopsis thaliana* line with deficiency in the histone variant H2AX was generated. This line showed neither sensitivity to genotoxic stress nor DNA repair changes but reduced AtATM-dependent, and AtE2F-mediated, transcriptional induction of the stress genes, AtR2-3b and AtRAD51. Interestingly, in *Nicotiana tabacum* E2F formed nuclear foci colocalizing with  $\gamma$ H2AX foci in an ATM-dependent manner. Loss of the transactivation domain of E2F reduced foci formation suggesting a role of E2F foci formation in the coupling of transcription to DNA repair.

The dynamic interactions of NHEJ complexes were the subject of the lecture given by **Dik C. van Gent** (University Medical Center Rotterdam). Using fluorescence resonance energy transfer (FRET), the heterodimer Ku70/Ku80 was seen to directly bind to DSBs and this binding was stabilized by DNA-PK<sub>cs</sub>, LigIV and XRCC4. Addition of ATP reduced the stability of the complex which was interpreted as promotion of DNA end processing after autophosphorylation of DNA-PK<sub>cs</sub>. Even though the C-terminal 14 amino acids of Ku80 are involved in the interaction with DNA-PK<sub>cs</sub>, deletion neither prevented DNA-PK<sub>cs</sub> binding nor kinase activation, did, however, abrogate DNA end processing by Artemis.

The role of HR and NHEJ in cellular protection against O6MeG was the subject of the lecture by **Wynand P. Roos** (University of Mainz). Cells defective in HR showed significantly increased sensitivity towards temozolomide compared to wildtype cells, which could be rescued by O6MeG methyltransferase (MGMT) overexpression.  $\gamma$ H2AX foci analysis indicated that DSBs arising from O6MeG processing were normally repaired by HR rather than NHEJ. As expected, HR defective cells also displayed massive chromosomal aberrations after temozolomide treatment. The collected data were interpreted as evidence for a recombinative bypass of persistent O6MeG:T mispairs during DNA replication.

*Xrs2* is the functional analogue of the human *NBN* gene in yeast and **Sylvia Steininger** (Helmholtz Center Munich) reported on the analysis of IR sensitivity in *Xrs2* mutants. Use of a novel plasmid reporter system indicated reduced gap repair efficiency but

normal accuracy. Crossover frequencies were strongly reduced in *Xrs2* mutants which was not corrected by additional mutation of the DNA helicases *Srs2* or *Sgs1*. For its repair function, neither the *Tel1* binding, BRCT nor FHA domains but the *Mre11* binding site was essential. The findings suggest that *Xrs2* has an early function in a HR pathway involving crossover for DSB repair.

### 3: Chromatin Structure and DNA Repair

Assembly, activation and inactivation of pre-replication complexes (pre-RCs) are accurately controlled to ensure that the whole genome is replicated exactly one time during each cell cycle ("license to replicate"). **Malik Lutzmann** (Institut of Human Genetics, Montpellier) presented the oncogene *Cdt1* as an important DNA licensing component, whose inappropriate presence results in re-replication and DNA damage. During the licensing reaction, the replicative helicases MCM2-7 bind to chromatin, a process which is dependent on MCM9 which in turn forms a stable complex with *Cdt1*. MCM9 is, therefore, a regulatory factor of *Cdt1* which opposes its other regulatory factor, geminin which inhibits loading of MCM2-7 onto replication origins.

In his lecture, **Christof Niehrs** (Deutsches Krebsforschungszentrum, Heidelberg) described a fascinating example of the links between DNA repair and DNA demethylation. Overexpression of the DNA damage response factor *Gadd45a* in *Xenopus* oocytes led to activation of plasmid reporter genes previously silenced by methylation. *Gadd45a* knockdown silenced gene expression and induced DNA hypermethylation. The mechanism of demethylation turned out to involve nucleotide excision repair (NER) leading to loss of methylated cytosine. *Gadd45a* directly interacted with XPG and demethylation required XP proteins.

The role of epigenetic modifications in skin cancer was the subject of the talk given by **Rüdiger Greinert** (Dermatologisches Zentrum Buxtehude). Histological analysis of UV exposed skin sections indicated larger fractions of euchromatin in cells retaining pyrimidine dimers. Flow cytometric analysis using antibodies against methylated histone H3 variants specific for euchromatin (H3K4me2) or heterochromatin (H3K9me3), showed that euchromatinisation is a rapid process after UVA-irradiation. The kinetics of pyrimidine dimer disappearance correlated with H3K4me2 decrease. Thus, chromatin relaxation, induced by changes in histone methylation may facilitate DNA repair processes.

Primary microcephaly in humans can be caused by biallelic mutations in the *MCPH1* gene. Cells from these patients exhibit premature chromosome condensation in early G2, which was the topic of the lecture by **Marc Trimborn** (Charité – Universitätsmedizin Berlin). Elucidation of a putative role of *MCPH1* in DNA repair was complicated by the fact that

patient cells behaved differently to cells after knockdown of MCPH1 expression. This was particularly true for CHK1 phosphorylation and G2/M checkpoint functions following IR which were normal in patient cells but defective after knockdown. Nevertheless, failure to decondense chromatin and increased chromosome breakage early after IR in patient cells indicated a possible function of MCPH1 in a G2-phase specific DSB repair process or in decondensation of damaged chromatin.

Using GFP-fused versions of LigIV and XRCC4 in live cell imaging, **Christian Mielke** (Heinrich-Heine-University Medical School, Düsseldorf) observed that LigIV was exclusively nuclear only when LigIV and XRCC4 were expressed from a bicistronic construct at equimolar levels. Knockdown of XRCC4 rendered LigIV almost exclusively cytoplasmic. From this, XRCC4 is clearly required for the functional localisation of LigIV in the nucleus.

**Paulius Grigaravicius** (Leibniz Institute for Age Research, Jena) also applied live cell imaging with GFP-tagged proteins to study the dynamics of Ku80, XRCC4 and nibrin after DSB-induction by a highly focused laser microbeam. XRCC4 and Ku80 were recruited rapidly to DSBs (55 seconds), followed by nibrin (75 seconds). Consistently, XRCC4 recruitment was normal in *NBN* null mutant mouse cells. Examination of endogenous protein in fixed cells substantiated these findings. Thus, although nibrin is generally considered to be a primary sensor of DSBs for both NHEJ and HR, XRCC4 was recruited to DSBs first.

### **Public Lecture**

The public lecture was given by **Stanton L. Gerson** (Case Western Reserve University, Cleveland) on the role of DNA repair in stem cell aging. Defects in DNA repair genes are critically detrimental to stem cell survival as was documented by S.L. Gerson for bone marrow reconstitution failure with MMR-defective cells. Perhaps the most impressive data S.L. Gerson shared with the audience was the linear relationship between spontaneous microsatellite instability in hematopoietic stem cells and individual age. The accumulation of mutations with age may reduce stem cell longevity and thus be a major determinant of the lifespan of an individual.

### **4: Telomeres / Double-Strand Break Repair II**

**Sascha Beneke** (University of Konstanz) described a new involvement of the poly-ADP-ribosylation system in the control of telomere length. Several members of the PARP family had been localized to telomeres, suggesting a physiological role in telomere maintenance. Human and hamster cells treated with the PARP inhibitor 3-aminobenzamide (3AB) showed a dose-dependent reduction in telomere length with time to 75% of untreated cells without altering 3'overhang stability or telomerase activity. The same effect was

achieved by siRNA knockdown of PARP-1 but not PARP-2 leading to the idea that PARP-1 specifically facilitates opening of telomeres in S-phase for telomerase.

**Predrag Slijepcevic** (Brunel University) introduced the integrative model of telomere maintenance. Knockout mice and methods such as Q-FISH indicated that at least 17 DNA repair proteins affect telomere maintenance. The integrative model attempts to explain the presence of these proteins at telomeres which are considered to be structures whose function is to prevent inappropriate repair of chromosome ends. Thus, telomere maintenance is an aspect of chromosome repair in the sense of restoration of chromosome function. DNA damage sensing, signalling and repair are closely associated with chromosome repair. In support of the concept were the findings in telomerase deficient mouse and human cells which also showed defects in the DNA damage response.

**Ilja Demuth** (Charité - Universitätsmedizin Berlin) studied the telomere accessory protein hSNM1B/Apollo, which has sequence similarity to the yeast *Pso2* gene implicated in DNA crosslink repair and which interacts with the telomere shelterin complex factor, TRF2. Live cell imaging showed that in addition to telomeric foci, hSNM1B accumulated within seconds after DSB-induction at the sites of DNA damage. hSNM1B knockdown led to attenuated ATM and ATR signalling after IR and UV, respectively. In line with the integrative model, hSNM1B has dual roles in the early DNA damage response and in telomere maintenance.

**Kathrin Lange** (Hannover Medical School) examined telomere length variation in cells from 48 patients with myelodysplastic syndrome (MDS) versus healthy controls using Telomere/Centromere Fluorescence in-situ Hybridization, which compares signal strengths from hybridized telomere with centromere PNA probes. The results showed that mean telomere length was significantly reduced in MDS patients without significant differences between cytogenetic subtypes but extensive shortening in aggressive MDS. It was proposed that telomere erosion plays a role in the formation of aberrant karyotypes with subsequent up-regulation of telomerase leading to telomere stabilisation in tumor cells.

Starting point for the experiments described by **Claudia Rübe** (Saarland University, Homburg/Saar) was the observation that 5% of patients undergoing radiotherapy have severe side effects which could be due to individual variation in the proficiency of DSB repair. Consequently, mice with pathological mutations in *ATM* or *Prkdc* (DNA-PKcs) or single nucleotide polymorphisms (SNP) in *Prkdc* were irradiated *in vivo* and various tissues examined by the sensitive  $\gamma$ H2AX assay. DSB repair deficiency was readily apparent for pathological mutations and measurable for *Prkdc* SNPs. Since blood lymphocytes irradiated both *in vivo* und *in vitro* showed similar repair capacities, this relatively simple assay might be

useful for identifying patients at risk before radiotherapy.

**Steffi Kuhfittig-Kulle** (University Children's Hospital Essen) studied the mechanism underlying different clinical presentations of neuroblastoma. Expression of the neurotrophin tyrosine kinase receptors, TrkA and TrkB, correlates with good and bad prognosis, respectively. Using the  $\gamma$ H2AX assay and *in vitro* DNA repair assays, it was found that neuroblastoma SY5Y cells expressing TrkA rapidly repaired IR-induced breaks, particularly by NHEJ. In contrast, repair in SY5Y cells expressing TrkB was slower and used the less accurate micro-SSA. XRCC4 was found to be up-regulated in SY5Y cells expressing TrkA suggesting that this might cause improved repair capacity and, thus, milder clinical progression.

## 5: Excision Repair

**Primo Schär** (University of Basel) provided evidence for the regulation of BER by SUMO modification. SUMO conjugation of the thymine DNA glycosylase (TDG) facilitated dissociation from the processed abasic site and degradation. Further downstream in BER, the affinity of XRCC1 for other BER proteins was altered by SUMO modification. Interestingly, the SUMO moiety turned out to be removed by a specific protease and transferred to TDG, a process dependent upon the assembly of the repair machinery. With XRCC1 being present at the DNA lesion before TDG, sequential SUMO-conjugation may, thus, coordinate BER.

**Bernd Epe** (University of Mainz) further delineated the role of 8-oxoguanine DNA glycosylase (Ogg1) in the main BER pathway for oxidative base damage. In the absence of Ogg1, PARP1, CSB, XPA and XPG contributed to a back-up pathway, as indicated by the accumulation of oxidative damage in double knockout mice. Lacl mouse derivatives revealed an increased rate of spontaneous mutations and of preneoplastic foci in the livers. Interestingly, oxidative stress or depletion of glutathione by treatment of cells with Buthionine sulfoximine retarded repair of oxidative base damage indicating thiol-dependency of BER (not NER). Importantly, human cells with a common variant of Ogg1, Ser326Cys, displayed slower repair kinetics of oxidative damage.

The crystal structure of the NER helicase XPD from the archaeobacterium *Thermoplasma acidophilum* was presented by **Caroline Kisker** (University of Würzburg). The protein possesses two RecA-like helicase domains and a 4Fe4S cluster domain forming a central hole with a diameter of 13Å. It was speculated, that a DNA strand passes through the hole and is checked for damaged bases, possibly through a pocket formed by highly

conserved amino acids into which only undamaged bases would fit. The positions of several mutations found in Xeroderma pigmentosum would support this model.

The tumor suppressor protein p53 was reported to transcriptionally regulate the NER genes *XPC* and *DDB2*. In his talk, **Gunnar Jahnke** (Technische Universität Berlin) used cell lines with reduced or absent p53 expression to study the removal of Benzo [a] pyrene diol epoxide (BPDE) DNA-adducts and UVC-induced pyrimidine dimers (CPD) by HPLC or quantitative fluorescence microscopy. Surprisingly, absence of p53 diminished repair of CPDs but not of BPDE adducts. Possible explanations could come from differences in lesion recognition or the numbers of lesions generated in the protocol used: 10,000 CPDs/10<sup>8</sup>b vs. 750 BPDE-adducts/10<sup>8</sup>b.

Testicular Germ Cell Tumors (TGCT) account for 1% of male malignancies and respond particularly well to cisplatin-based combination therapy. **Svetlana Usanova** (University of Mainz) found reduced ERCC1-XPF expression levels in TGCT in comparison to bladder tumours. This protein complex has been implicated in the repair of cisplatin intrastrand and interstrand crosslinks. Using an intrastrand-specific antibody indicated that NER proceeded with comparable kinetics in bladder tumour and TGCT cell lines after cisplatin treatment. In contrast, repair of interstrand crosslinks, measured by the Comet assay, was clearly defective in TGCT cell lines. Overexpression of ERCC1 and XPF in TGCT cell lines improved crosslink repair and even cell survival after cisplatin treatment.

**Martina Bauer** (University of Mainz) focused on repair of alkylation damage in blood monocytes. When compared to dendritic cells (DC), their precursors, monocytes, were more sensitive to temozolomide. This hypersensitivity was not related to O6-methylguanine since MGMT was actually down-regulated from monocytes to DCs. Further gene expression analysis revealed that XRCC1 and LigIII $\alpha$  were not expressed in monocytes but became up-regulated during maturation to DCs. Monocytes, stimulated to express XRCC1 and LigIII $\alpha$  by treatment with IL-4 and GM-CSF *in vitro* showed increased resistance to alkylating agents.

## 6: Replication Fork Arrest

**Ian Hickson** (University of Oxford) focused on BLM, which is part of an evolutionary conserved protein complex together with topoisomerase III $\alpha$  and two other factors, RMI1 and RMI2. This BTR complex catalyzes the dissolution of Holliday junctions, thereby preventing sister chromatid exchanges as well as chromosome segregation abnormalities in mitosis. BLM and the other BTR complex members were observed to localize to anaphase bridges during mitosis. Strikingly, Bloom's syndrome (BS) cells showed excessive anaphase bridging. Immunofluorescent staining of BLM detected a class of ultrafine anaphase bridges,

which were not detectable by DAPI. These bridges were identical to the previously described PICH threads and in part contained FANCD2. FANCD2-associated bridges were enriched at fragile sites, where gaps, breaks and chromosomal rearrangements cluster after replication inhibition. Incomplete replication at these sites with late completion in S-phase could require decatenation by the BTR complex in mitosis.

**Ulrich Hübscher** (University of Zürich-Irchel) elucidated an important role of PCNA and RP-A in determining functional hierarchy among the different DNA polymerases during bypass of 8-oxo-guanine (8-oxo-G) lesions. 8-oxo-G is a potentially mutagenic lesion because of its miscoding character. Among the six polymerases belonging to classes B, Y or X analysed *in vitro*, a major and specific effect was shown for DNA polymerases  $\lambda$  and  $\eta$ . Addition of PCNA and RP-A specifically inhibited the misincorporation of dATP opposite 8-oxo-G, resulting in a 1,200-fold more correct incorporation of dCTP by polymerase  $\lambda$  and a 68-fold more correct incorporation by polymerase  $\eta$ . DNA polymerase  $\lambda$  was found to be stabilised by Cdk2-mediated phosphorylation, particularly in late S phase, enabling polymerase  $\lambda$  to participate in DNA repair processes during and after S phase.

**Gerhard Fritz** (University of Mainz) analysed cell lines defective in DNA damage recognition, signalling or repair with respect to a contribution of DNA damage to the activation of the stress kinases SAPK/JNK. DNA-PK<sub>CS</sub> and CSB were identified to promote late ( $\geq 2$ h) SAPK/JNK activation following MMS treatment. However, early phosphorylation of SAPK/JNK ( $< 2$ h) in response to UVC irradiation was evoked by blockage of DNA replication.

**Kerstin Borgmann** (University Medical School Hamburg-Eppendorf) observed a decrease in DSB repair by HR, an overall decrease of DSB repair as measured by  $\gamma$ H2AX foci formation, an increased rate of chromosomal aberrations in G2 phase leading to a pronounced G2-arrest and increased sensitivity to the DNA interstrand crosslinking agent Mitomycin C (MMC) as a consequence of inducible RAD51 overexpression in U2OS cells. These cells, thus, showed phenotypic similarities to cells from Fanconi Anemia (FA) patients and indeed, expression of several FA-genes was found to be reduced at the RNA level.

**Kirsten Anke Schürer** (University of Oxford) reported on the identification of a new human FANCM isoform (FANCM-669), which represents the N-terminal portion of FANCM, contains a DEAH box and shows sequence similarity to MPH1 of *S. cerevisiae* and the mfh1 and mfh2 proteins of *S. pombe*. FANCM-669 translation is terminated by a stop codon in intron 11 of a FANCM splice variant and the protein was detectable in western blots from human whole cell extracts. As shown by *in vitro* analysis of the purified protein, FANCM-669

is a DNA-dependent ATPase which can disrupt DNA D-loops, possibly arising during error free bypass of DNA lesions.

The human tyrosyl-DNA phosphodiesterase (TDP1) cleaves the phosphodiester bond between a covalently stalled topoisomerase I and the 3' end of DNA. A homozygous H493R mutation was shown to be the basic defect in patients with the hereditary neurological disorder spinocerebellar ataxia with axonal neuropathy (SCAN1). **Heidrun Interthal** (University of Edinburgh) showed that H493R is not a loss-of-function mutation as earlier proposed. Instead, mutant TDP1 retained residual activity. As a consequence TDP1 itself would form a new protein-DNA complex which accumulates and likely contributes to the SCAN1 phenotype, a hypothesis which was strongly supported by the analysis of Tdp1 knock out mice.