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ABSTRACT

The First joint meeting of the German DGDR (German Society for Research on DNA Repair) and the French SFTG (French Society of Genotoxicology) on DNA Repair was held in Toulouse, France, from September 15 to 19, 2007. It was organized by Lisa Wiesmüller and Bernard Salles together with the scientific committee consisting of Gilbert de Murcia, Jean-Marc Egly, Frank Grosse, Karl-Peter Hopfner, Georges Iliakis, Bernd Kaina, Markus Löbrich, Bernard Lopez, Daniel Marzin and Alain Sarasin. This report summarizes information presented by the speakers (invited lectures and oral communications) during the seven plenary sessions, which include (1) excision repair, (2) DNA repair and carcinogenesis, (3) double-strand break repair, (4) replication in repair and lesion bypass, (5) cellular responses to genotoxic stress, (6) DNA repair machinery within the chromatin context and (7) genotoxicology and testing. A total of 23 plenary lectures, 32 oral communications and 66 posters were presented in this rather intense 4 days meeting, which stimulated extensive discussions and highly interdisciplinary scientific exchanges among the ~250 participants.

This DNA Repair meeting was hosted by the Faculty of Pharmacy at the University of Toulouse (South of France) and initiated a new German–French conference series to generate a platform for intensified scientific exchange between both countries through gatherings every 2 years; next meeting will be held in Konstanz (Germany) September 20–23, 2009. This meeting comprised seven plenary sessions each embedded in lively poster discussions and started with a keynote lecture presented by André Nussenzweig whose name, as he himself noticed, is a pure example of German (Nussenzweig)–French (André) fusion! As was reflected by the broad international participation, scientists from other countries were very welcome and enthusiastically contributed to the success of this meeting. This report summarizes information presented by speakers during the different sessions and includes bibliography related to their talks.

Keynote lecture

In his keynote lecture, André Nussenzweig (NIH, Bethesda, USA) addressed the issue of programmed DNA damages, namely those generated during antigen receptor diversification reactions in lymphocytes such as during V(D)J

recombination. This process is initiated by RAG1/2 (the proteins encoded by recombinase-activating genes 1 and 2), which introduce double-stranded DNA breaks (DSBs) at recombination signal sequences (RSSs). After being stimulated with antigen, mature B cells undergo two additional antigen receptor-diversification reactions called class-switch recombination (CSR) and somatic hypermutation (SHM) that are both initiated by an activation-induced cytosine deaminase (AID) [1]. André Nussenzweig outlined his recent findings with AID mutant mouse models, which directly linked CSR and *c-myc/IgH* translocations found in Burkitt's lymphoma and provided evidence that AID is required to trigger CSR via DNA break formation [2]. He further demonstrated that ATM (Ataxia telangiectasia mutated) plays a critical role in preventing the persistence and propagation of chromosome breaks produced by failed end joining during V(D)J recombination [3]. These RAG1/2 endonuclease-dependent breaks, which are detectable as terminally deleted chromosomes in ATM-deficient lymphocytes, persist for weeks *in vivo* and are transmitted over generations *in vitro*. These persistent DNA ends may become substrates for Ku-independent end joining pathways and, therefore, for translocations involving oncogenes with recurrent breakpoints [3]. This work revealed two complementary functions of ATM in suppressing chromosomal aberrations and malignant transformation in lymphocytes undergoing

diversification of antigen receptor genes: a role in counteracting error-prone DSB repair of chromosomal breaks and an ATM checkpoint that eliminates cells with DSBs.

1. Excision repair

The session began with Serge Boiteux (CEA, Fontenay-aux-Roses, France), who focused on the repair of oxidative DNA damage. In this respect, 7,8-dihydro-8-oxoguanine (8-oxoG) is a pathobiologically highly relevant lesion, since it has been suggested that the capacity of skin cells to repair 8-oxoG may be a key parameter in the carcinogenic effect of UVA radiation in humans [4]. In *S. cerevisiae*, the main defence against the mutagenic effect of 8-oxoG (mainly GC to TA transversions) is the base excision repair (BER) pathway which is initiated by the Ogg1 protein (8-oxoG DNA N-glycosylase/AP lyase) [5]. Analysis of yeast strains mutated in *Ogg1* and/or *Msh6*, *Rad30*, *Rad18*, and *Rad6* indicated that DNA mismatch repair (MMR), translesion synthesis (TLS) by polymerase η (*Pol\eta*; *Rad30* gene product) and post-replication repair (PRR; initiated by *Rad18* and *Rad6*) synergistically cooperate with Ogg1 to prevent the mutagenic effect of 8-oxoG [6]. For comparison, the protective effect of Rad51-dependent homologous recombination (HR) was additive. From his data, Serge Boiteux proposed a hierarchical network that protects cells from the mutagenic action of oxidative DNA damage. In this model, 8-oxoG leads to a mispaired G^0/C that is removed by Ogg1 in the course of a BER process restoring G/C; if Ogg1 is absent, Pol δ replication past 8-oxoG produces a G^0/A excised by specialized MMR. The removal of adenine opposite 8-oxoG generates an ssDNA gap recognized by PCNA and association of the Rad6–Rad18 complex, which in turn mediates PCNA ubiquitination. Ubiquitinated PCNA recruits Pol η , which enables error-free TLS across 8-oxoG, restoring G^0/C , i.e. the substrate of Ogg1. In the absence of Rad18 or Rad6, unmodified PCNA preferentially recruits Pol δ at excision gaps and promotes the mutagenic incorporation of adenine; in the absence of MMR, cells accumulate GC to TA transversions [6].

Valérie Schreiber (CNRS, Illkirch, France) reviewed the multiple functions of some of the 17 PARP family members. PARPs mediate poly(ADP-ribosylation), a post-translational modification of proteins involved in DNA repair, transcription, mitotic segregation, telomere homeostasis and cell death [7]. PARP-1, the founding member of the family, and PARP-2 (discovered as a result of the presence of residual DNA-dependent PARP activity in embryonic fibroblasts derived from PARP-1-deficient mice) are the only known members whose activity is stimulated by DNA strand breaks. PARP-1/2 targets are mainly involved in chromatin structure and DNA metabolism such as histones (in particular H1 and H2B), DNA repair proteins, topoisomerases and transcription factors. PARP-1 is mostly described as a molecular sensor of DNA breaks and exhibits a more direct DNA repair function during BER via recruitment of XRCC1 to the damaged site. Although *Parp-2*^{-/-} mice also display genomic instabilities after DNA damage and a defect in BER, PARP-2 does not recruit XRCC1. Additional PARP-2 functions have recently been identified in spermatogenesis [8], T cell development [9] and fat acid metabolism. The impact of poly(ADP-ribosylation) reactions may indeed be considerably

extended by (i) the diversity of functional PARP domains, (ii) the variety of subcellular localizations and (iii) the numerous, newly discovered biological functions. Furthermore, PARG (the poly(ADP-ribose) degrading enzyme) controls the poly(ADP-ribose) levels. The fact that PARG inactivation in human tumor cells caused mitotic defects and X-ray sensitivity suggests that PARG must be considered a potential target for radiotherapy.

Jean-Marc Egly (CNRS, Illkirch, France) addressed the role of human transcription/repair factor TFIIH in nucleotide excision repair (NER). TFIIH consists of ten subunits: XPB, XPD, p62, p52, p44, p34, p8/TTDA form the core complex and cdk7, MAT1, cyclin H form the cdk-activating kinase (CAK) subcomplex in the centre. Mutations in XPB, XPD, or p8/TTDA have been linked to Xeroderma Pigmentosum (XP), Cockayne Syndrome (CS) and Trichothiodystrophy (TTD). Employing an NER assay with immobilized, cisplatinated DNA template and isolated NER components including proteins for resynthesis and ligation, Jean-Marc Egly and co-workers were able to completely reconstitute the NER reaction *in vitro* [10]. Using either wild type or mutated recombinant proteins, they showed that the NER factors were sequentially joining and leaving the DNA in a ballet similar to what has been observed by confocal microscopy in the cell. More specifically, ATP was required to promote association of XPA, RPA (replication protein A), XPG and XPF to preexisting XPC/TFIIH/DNA-complexes [10]. Jean-Marc Egly and co-workers also suggested a new mechanism in which the helicase activity of XPB is not used for the opening and repair of damaged DNA, which instead is driven by its ATPase activity in combination with the helicase activity of XPD [11]. Vice versa, for transcription the XPB but not XPD helicase is necessary. These studies provide new clues to puzzling clinical observations such as the absence of mutations in the helicase motif of XPB and in p52, which is necessary for the XPB ATPase.

In his talk about repair of oxidative DNA lesions, Bernd Epe (University of Mainz, Germany) stressed the fact that 8-oxoG probably is the most mutagenic lesion in mammalian cells. Work from his group indicated that, although 8-oxoG is removed predominantly via BER, backup pathways exist [12–14]. This backup repair depends on CSB, XPA and PARP-1 (epistatic relationship) but not on CSA, XPC or p53, and does not require Ogg1 activity. Thus, residual 8-oxoG repair observed in *Ogg1*^{-/-} cells is absent in *Ogg1*^{-/-}/*Parp-1*^{-/-} and *Ogg1*^{-/-}/*Csb*^{-/-} cells, consistent with a two-fold higher level of spontaneous mutation frequencies in the livers of the double knock-out mice additionally carrying the lac I reporter of big Blue mice. Since PARP-1 appeared to be required for an efficient block of transcription in this process, Bernd Epe suggested the existence of an as yet unknown repair pathway in which stalled RNA Pols could act as a trigger for overall repair of oxidative DNA base modifications.

This opening session ended with four proffered papers. First, Marie-Pascale Doutriaux (CNRS, Orsay, France) reported on the isolation of an Arabidopsis DUT1 cDNA encoding a dUTPase activity. RNAi-mediated *DUT1* gene silencing led to defective flower opening. Knock-down in *Ung*^{-/-} plants (devoid of uracil DNA glycosylase) caused premature death, suggesting toxicity of uracil accumulation. Then, addressing PARP-mediated responses to genotoxic insults, Jörg Fahrner (University of Konstanz, Germany) showed by electrophoretic

mobility shift and Surface Plasmon Resonance assays that poly(ADP-ribose) (PAR) specifically, with high affinities in the nM range and in a chain-length dependent manner interacts with p53 and XPA, suggesting the existence of a “PAR-code”. Then, Elisa Ferrando-May (University of Konstanz, Germany) newly introduced DEK, an abundant and ubiquitous chromatin factor, originally described as part of the DEK-CAN fusion protein associated with acute myeloid leukemia cases. In a proteomic screen of apoptotic nuclei, Elisa Ferrando-May and co-workers identified DEK as a target of decreased phosphorylation in early apoptotic T cells [15]. Here, she showed that DEK is an efficient acceptor of PAR, which in turn negatively influences its DNA binding activity and its ability to introduce topological changes in DNA templates. The analysis of chemotherapeutic drug sensitivities, DNA breakage and signaling after DEK knock-down unveiled an exciting link between DEK and PAR-mediated DNA damage responses involving ATM. Lastly, Martijn Luijsterburg (University of Amsterdam, The Netherlands) reported on his *in vivo* analyses of Damage DNA binding protein 2 (DDB2), which is essential for the repair of cyclobutane pyrimidine dimers (CPDs) and is part of the cullin 4A (CUL4A)-based E3 ubiquitin ligase. Using fluorescent fusion proteins combined with life cell microscopy after bleaching, he showed that the bulk of DDB2 binds to and dissociates from UV-induced lesions independently of the damage-recognition protein XPC. The DDB2-containing E3 ligase complex was bound to many more damaged sites than XPC, suggesting that there is little physical interaction between the two proteins. From his data, Martijn Luijsterburg proposed a model according to which DDB2 binds the lesion, then, gains ubiquitin ligase activity directed towards histones H3 and H4 and prepares UV-damaged chromatin for the assembly of the NER complex [16].

2. DNA repair and carcinogenesis

Since DNA repair systems and cell cycle checkpoints are critical components of the cellular response to DNA damage, the presence of genetic variants in these pathways was predicted to modulate cancer risk. Addressing this issue, Janet Hall (INSERM, Orsay, France) reported on a large-scale multicenter epidemiological study on tobacco-related cancers, enrolling 2200 lung, 1000 head and neck, 1000 kidney cancer cases and 3000 controls in 6 countries of central Europe (IARC). From the 27 variants in 17 repair genes investigated, she focused her talk on the single nucleotide polymorphism (SNP) in *Ogg1* (326C) [22] and variant p53PIN3 in *TP53* (for details see Virginie Marcel below), which were found to be associated with increased risk of lung cancer. Consistent with a functional defect, a two-fold difference in *Ogg1* activity was measured in lymphoblastoid cell lines from homozygous *Ogg1* 326C versus 326S carriers. In contrast, for the variant allele *CHEK2* 157I a lower incidence of lung and upper aero-digestive cancers was noted. Janet Hall further showed that variant *ATM* 1054R, which confers an approximately two-fold elevated prostate cancer risk [17], is functionally linked to genomic instabilities, an altered radiation response and slower DNA damage removal. Interestingly, a strong gene-environment interaction has been noted for some of these variants, particularly regarding smoke exposure

[18,19]. There is no doubt that characterization of the molecular mechanisms underlying these associations will help to better understand the environmental factors influencing carcinogenesis.

Through a DNA microarray profiling approach involving the analysis of >38,000 genes in tissues from 176 independent primary melanomas from patients with a median follow-up of 8.5 years, Alain Sarasin (CNRS, Villejuif, France) and co-workers established a gene expression signature that identifies primary malignant melanoma with high metastatic potential (and hence poor clinical outcome) [20]. Their data further unveiled that overexpression of DNA repair genes, particularly those involved in DSB repair and surveillance of replication forks, is associated with metastasis and poor patient survival [21]. Alain Sarasin concluded that, while genetic instability is well known to be necessary for the initial transition from normal to tumor cells, subsequently, some type of genetic stabilization may be needed in order to allow the establishment of a population of sufficiently stable primary tumor cells to enable invasion and distant metastasis.

This short session ended with two proffered papers. First, by comparing the level of basal and oxidative DNA damage and the kinetics of removal in peripheral blood lymphocytes from 30 gastric cancer patients versus 30 healthy individuals, Janusz Blasiak (University of Lodz, Poland) observed an association between gastric cancer occurrence, impaired repair of oxidative DNA lesions and the *Rad51* 135C, but not the *Ogg1* 326C gene polymorphism [22]. Then, Virginie Marcel (CIRC, Lyon, France) addressed the role of p53 isoforms. She observed that homozygosity of the p53PIN3 polymorphism, a common 16 bp duplication in intron 3 of *TP53* mostly coupled with polymorphism 72P, not only decreases the total level of *TP53* mRNA [23] but concomitantly also changes the balance between full-length and spliced forms of *TP53*. She showed that G-quadruplex structures overlap the p53PIN3 and that disruption of these quadruplexes favours retention of intron 2. This suggested that these structures modulate splicing, which could explain increased cancer susceptibility associated with p53PIN3.

3. Double-strand break repair

DSBs are considered the most severe lesions: left unrepaired, DSBs can result in apoptosis or mitotic cell death; repaired incorrectly, they can lead to carcinogenesis through mutagenic genome rearrangements. Produced exogenously by ionizing radiation (IR) or chemicals, but also endogenously during DNA replication or programmed processes such as V(D)J recombination and meiotic exchange, DSBs are usually repaired by either nonhomologous end joining (NHEJ) or homologous recombination (HR). While NHEJ allows fast but error-prone repair during the entire cell cycle, the slower but high-fidelity HR pathway is mostly restricted to S and G2 phases [24]. To get further insight into the partitioning of these two DNA repair pathways, Markus Löbrich (Darmstadt University, Germany) and colleagues analysed DSB repair kinetics in different NHEJ and HR mutants using γ H2AX foci analysis in combination with cell cycle marker and *Rad51* staining. Formation and disappearance of γ H2AX foci has recently pro-

vided an extraordinarily sensitive technique to monitor DSB introduction and repair [25]. They found that ATM activates an Artemis-dependent end-processing mechanism, which is required for the repair of a sub-fraction (approximately 10%) of IR-induced DSBs [26,27]. More precisely, epistasis-like studies with specific inhibitors of ATM and DNA-PKcs along with siRNA-mediated down-regulation of ATM/Artemis and other NHEJ or HR components revealed that ATM and Artemis contribute to HR in G2. Hence, these two proteins represent components of NHEJ in G1 and HR in G2, a striking conclusion which was further supported by the finding that the ATM/Artemis-subpathway involves DNA-PK but not BRCA2 in G1 and BRCA2 but not DNA-PK in G2.

Nbs1, mutated in NBS (Nijmegen Breakage Syndrome), together with Mre11 and Rad50 forms an evolutionary conserved protein complex (MRN) which acts as a DNA damage sensor and transducer and plays a critical role in regulating DNA repair, cell cycle checkpoints and apoptosis. However, in mammals these functions of Nbs1 have not fully been elucidated due to the lethal phenotype of cells and mice lacking Nbs1. Hence, Zhao-Qi Wang (Fritz Lipmann Institute, Jena, Germany) and co-workers generated conditional knock-out mice, in which a null mutation of *Nbs1* can be introduced in specific tissues and cell types using the Cre-loxP technique [28]. Cells lacking Nbs1 displayed reduced HR and increased NHEJ-mediated sequence deletions along with attenuated chromatin recruitment of Rad50, Mre11, Rad51 and Brca1, but not Ku70 [29]. Upon *Nbs1* knock-out in the central nervous system, mice showed retarded growth, microcephaly, ataxia and defects in cerebellum development. In neuroprogenitors, impaired ATR signaling and hyperactivation of the ATM-p53 pathway due to the persistence of chromosomal breaks was noted. Consistently, the morphology of Purkinje cells and the size of the cerebellum were rescued after crossing with *p53*^{-/-} mice [30]. Since NBS patients also suffer from severe immunodeficiency, Zhao-Qi Wang and co-workers generated mice carrying the *Nbs1* deletion in B lymphocytes, enabling them to detect hypersensitivity to IR, checkpoint and proliferation defects and impaired IgG CSR. Knock-out in T lymphocytes caused a severe reduction of double positive (CD4⁺/CD8⁺) as well as single positive (CD4⁺/CD8⁻ and CD4⁻/CD8⁺) T cells associated with impaired TCR β rearrangements. These models finally provided *in vivo* evidence for the role of MRN in the DNA damage response as well as in neuronal and immunological development.

George Iliakis (University of Duisburg, Essen Medical School, Essen, Germany) presented his intriguing data on the existence of a non-canonical NHEJ mechanism. Thus, as a first approximation NHEJ can be subdivided into two pathways: the DNA-PK-dependent NHEJ pathway (D-NHEJ; sometimes also named C-NHEJ for "classical" NHEJ) and a backup pathway (B-NHEJ; sometimes also named A-NHEJ for "alternative" NHEJ) [31]. While D-NHEJ is responsible for a large proportion of the fast component of DSB rejoining and requires the activities of DNA-PK and DNA Ligase IV/XRCC4, B-NHEJ rather corresponds to slow rejoining, however, is active in DT40 chicken cell mutants of the *Rad52* epistasis group, i.e. does not depend on HR. Indeed, B-NHEJ is responsible for the majority of end joining events in mammalian cells with defects in factors required for D-NHEJ. Biochemical experiments with cell fractions and

purified proteins as well as cell-based NHEJ measurements after knock-down of specific components indicated that B-NHEJ utilizes Ligase III in complex with XRCC1 and PARP-1 and revealed an end holding activity of histone H1 in this process [32,33].

Jean-Pierre de Villartay (INSERM, Paris, France) focused his talk on the repair of DSBs generated by V(D)J and CSR processes, two programmed somatic rearrangements occurring during development and the maturation of the immune system. When performing a survey of patients suffering from severe combined immunodeficiency (SCID) and other developmental anomalies such as microcephaly, Jean-Pierre de Villartay and co-workers newly identified DNA repair factors including Artemis [34] and Cernunnos [35] (also known as XLF [36]). Cernunnos-XLF physically interacts with the XRCC4/Ligase IV complex, but its specific role in NHEJ remains to be deciphered [36,37]. Furthermore, although XRCC4 is essential for the resolution of DSBs during V(D)J recombination, its role in CSR had not been clarified. Using conditional knock-out mice to bypass embryonic lethality, his group showed that Artemis is dispensable for CSR. B lymphocyte restricted deletion of XRCC4 led to an average two-fold reduction of CSR [38]. Additionally, they observed an increase of short microhomologies at the junctions in XRCC4 deleted cells. These results connected XRCC4 and hence NHEJ to CSR and concomitantly supported the concept of alternative NHEJ during CSR, as documented by others [39,40].

Karl-Peter Hopfner (Gene Center, Munich, Germany) provided insight into the chemistry of translesion synthesis (TLS) at atomic resolution. In his lecture he described biochemical and structural studies on Pol η . This Pol enables error-free replication across cyclobutane pyrimidine dimers and crosslink lesions such as 1,2-d(GpG) cisplatin adducts (Pt-GG) and thereby survival after UV exposure and resistance against cisplatin-based chemotherapy. Karl-Peter Hopfner showed that bypass of the 3'dG is accurate, whereas A or C are incorporated across the 5'dG, which causes a severe distortion. His structural studies of the Pt-GG Pol η complex provided a molecular basis for the previously postulated induced fit. Thus, two different structures in the asymmetric unit unveiled that nucleotidyl transfer requires the DNA to rotate into the active conformation. Mutation of the highly conserved arginine 73 to leucine further showed that R73 forms part of a conserved clamp that positions and activates dNTP for scanning. These and future studies with more bulky platin compounds may lead to the development of new cisplatin derivatives that may overcome tumor resistance in cisplatin chemotherapy [41].

The PI3K/AKT pathway plays a central role in a variety of cellular processes including cell growth, proliferation and survival. Notably, many of the transforming events in breast cancer are a result of enhanced signaling of the PI3K/AKT pathway [42]. Since AKT1 phosphorylates the breast tumor suppressor Brca1 *in vitro*, Bernard Lopez (CNRS-CEA, Fontenay-aux-Roses, France) asked the question whether AKT1 has an impact on HR. Systematic analyses employing different techniques including life cell imaging unveiled that AKT1 represses IR- and I-Sce I-induced HR through cytoplasmic retention of Brca1 and Rad51 proteins. This process required the AKT1 effector and transcription factor FOXO1 for which physical interactions with Brca1 were also revealed. Bernard

Lopez proposed a model in which phosphorylation of FOXO1, but not Brca1, causes cytoplasmic retention and concomitantly drags its partners Brca1 and Rad51 to the cytoplasm. Highlighting the clinical relevance of these observations, immunohistochemical analysis indicated a preferential cytoplasmic localization of Brca1 in sporadic breast cancer tissue. This study suggests a role of AKT1-dependent HR regulation involving Brca1 and Rad51 in the formation of breast cancer without Brca1 mutation.

This quite intensive session was complemented by a series of highly interesting proffered papers. First, Susanne Gatz (University Children's Hospital, Ulm, Germany) provided evidence for a novel role of the natural plant constituent resveratrol in the control of DSB repair processes, which is exerted independently of its growth or death regulatory effects [43]. Using patient cell lines carrying distinct mutations, gene knock-down and chemical inhibition of distinct DSB repair factors, she established an ATM-p53-dependent pathway of HR inhibition and ATM/ATR-Nbs1-dependent inhibition of microhomology-mediated NHEJ by RV. Susanne Gatz proposed that activation of ATM and/or ATR is a central effect of RV, which could at least partially explain the chemopreventive effects in animal cancer models [44]. Applying the powerful tool of the enzymatically prepared, genome-wide RNAi library from the Buchholz lab [45], Mikolaj Slabicki (Max Planck Institute, Dresden, Germany) performed a screen for human genes involved in HR after cleavage by I-Sce I meganuclease. He identified 55 genes including Rad51, Brca1 and SHFM1 and 12 genes with unknown function. A highly interesting candidate shows structural homology to bacterial RecG. Serge Candéias (INSERM, Grenoble, France) reported on his surprising findings indicating that during V(D)J recombination junctional diversity is created not only at coding but also signal joints. Comparison of knock-out mice provided evidence for the involvement of terminal deoxynucleotidyl transferase (TdT) and Artemis in this process. Pei-Yu Wu (CNRS, University of Toulouse, France) elaborated on his mechanistic investigations regarding Cernunnos-XLF. He showed that in response to DSBs Cernunnos-XLF is phosphorylated by DNA-PK and, independently of phosphorylation, recruited to the chromatin, which mostly relies on Ligase IV/XRCC4. Vice versa Cernunnos-XLF is not required for the recruitment of the NHEJ core components [46]. Applying EGFP-based reporter constructs and junction DNA sequencing, Wael-Yassin Mansour (University Medical School, Hamburg, Germany) showed that loss of Ku entails a slight NHEJ decrease with a five-fold increase of deletions and of homology-directed DSB repair events in hamster cells. Unexpectedly, knock-down of Rad51 caused an increase of single-strand annealing (SSA) rather than a decrease of gene conversion. Pierre-Olivier Mari (Erasmus MC, Rotterdam, The Netherlands) introduced the method of multiphoton laser-induced DSB generation and fluorescence recovery after photobleaching (FRAP) to study the interdependence of NHEJ factors. Surprisingly, recruitment of XRCC4 turned out not to depend on the presence of DNA-PKcs. Altogether, his results indicated that core NHEJ recruitment to DSBs is fast, reversible, and takes place throughout the cell cycle [47]. Robert Schiestl (University of California, Los Angeles, USA) provided evidence for a mechanism, which is triggered by both IR-induced and targeted

DSBs and stimulates microhomology-mediated NHEJ at other genomic sites in yeast and mammalian cells [48]. Catherine Muller (CNRS, University of Toulouse, France) made the interesting observation that under hypoxic conditions, when replication forks stall and lead to DSB formation, DNA-PK is activated and protects HIF-1 α from proteasomal degradation by the von-Hippel Lindau pathway. The acute DNA damage response to hypoxia may, thus, mediate adaptation of cells to this microenvironmental stress. Addressing the influence of distinct phosphorylation events on direct DNA repair-regulatory functions of p53, Anja Restle (University of Ulm, Germany) showed that ATM/ATR-mediated phosphorylation at N-terminal sites enables wild type, but not mutant p53, to downregulate homologous DSB repair. Conversely, oncogenic p53 mutants were found to activate topoisomerase I-stimulated HR in a manner depending on phosphorylation at S315 by cyclin-dependent kinase 2 (Cdk2)/Cyclin A1. These data reveal that p53 mutants have lost the balance between activation and surveillance of HR, which results in a net increase of mutagenic DNA rearrangements and, therefore, provide new clues to genome destabilization by gain-of-function p53 mutants. Sharon Cantor (University of Massachusetts, Worcester, USA) summarized her latest data on the Fanconia anemia (FA) gene product FANCD1 (also called Brca1 or Brip1), which has been linked to breast cancer through its direct interaction with Brca1. She newly demonstrated that in addition to Brca1, FANCD1 binds to the MutL α complex. Reconstitution of FA-J patient cells with site-specific FANCD1 mutants showed that the helicase and MutL α binding activities are necessary to revert DNA interstrand crosslink (ICL)-induced sensitivity and prolonged G2/M arrest [49]. Loss of Brca1 binding, like FANCD1 suppression, led to reduced HR and activation of ICL repair bypass mechanisms via NHEJ or TLS, suggesting that Brca1 binding is highly important for FANCD1's tumor suppressor functions in the maintenance of genome stability. Simone Siehler (Universitätsfrauenklinik, Ulm, Germany) applied an EGFP based assay for the analysis of different DSB repair mechanisms on seven human cellular systems, differing in the MLH1 status. She found that MLH1, and its complex partners PMS1 and PMS2, downregulate gene conversion, particularly between non-identical sequences. Unexpectedly, hMSH2, but also ATM and BLM were dispensable, suggesting the involvement of an as yet unknown component in MutL-dependent HR surveillance. Finally, Carlos Menck (University of Sao Paulo, Brazil) investigated the impact of UV photoproducts in apoptosis induction in confluent and proliferating human primary fibroblasts. He observed strong p53 activation in both cell types but a reduced UV-induced apoptosis in confluent cells that was not associated with DNA damage removal.

4. Replication in repair and lesion bypass

SHM, which diversifies Ig genes during the immune response, is triggered by AID, which deaminates cytidines into uracils at the Ig locus. This initial lesion is further processed by TLS DNA Pols. In mice and man this leads to approximately 50% A/T base mutations, whereas in DT40 chicken cells, due to a high Ung-dependency, mainly G/C mutations are found. Via

analysis of single and double knock-out mice, Claude-Agnès Reynaud (INSERM, Paris, France) and co-workers established the critical role played by Pol η in A/T mutagenesis and showed that Pol κ serves as backup Pol [50,51]. She further demonstrated that Msh2 substantially contributes to the elevated mutation frequency by recruitment of Pol η and by prevention of Ung from performing error-free repair during SHM.

DNA lesions that could stall the replication machinery can be bypassed by TLS, which uses specialized, damage-tolerant DNA Pols. However, since their tolerance for distorted templates makes these enzymes less accurate, even on undamaged DNA, all organisms have control systems that keep TLS Pols in check and prevent their unrestrained activity in the absence of damage. Posttranslational modifications by ubiquitin and SUMO are part of this effective means of control. In the model system budding yeast, ubiquitin and SUMO compete for modification of proliferating cell nuclear antigen (PCNA). Monoubiquitination of PCNA facilitates the replicative bypass of DNA lesions by recruiting damage tolerant Pols, while deubiquitination of PCNA is a possible safeguard mechanism against the mutagenic effect of these Pols. More precisely, Helle Ulrich (Clare Hall Laboratories, London, UK) showed that, while monoubiquitination at K164 induces mutagenic lesion bypass, polyubiquitination at K164, involving K63 linkage, facilitates error-free damage avoidance that makes use of the genetic information encoded by the undamaged and homologous sister chromatid. In addition, sumoylation of PCNA at K127 and to a lesser extent at K164 targets the helicase Srs2 to active replication forks and thereby inhibits accumulation of Rad51 and unscheduled exchange processes [52–54]. Investigating the cellular signals required for PCNA ubiquitination *in vivo*, Helle Ulrich and colleagues found that the modification is induced by stretches of ssDNA associated with stalled replication forks. Her group finally also elucidated the mechanism by which the E3 ubiquitin ligase is targeted to PCNA, namely via recruitment of Rad18 to ssDNA by RPA.

In the proffered paper selected for this session, Heinz Peter Nasheuer (National University of Ireland, Galway, Ireland) presented biochemical and life cell imaging data on RPA1, a protein complex essential for eukaryotic DNA replication, repair and recombination as well as damage signaling. RPA1 consists of three subunits, p70, p32 and p14 and preferentially interacts with ssDNA. p32 is phosphorylated on serines 4 and 8 in response to UV and IR. Therefore, all three subunits plus RPA32 Δ N, a mutant lacking the N-terminus, were fused to GFP [55]. Excluding a major role of phosphorylation, both GFP-RPA32 and GFP-RPA32 Δ N localized to foci after UV and IR, although some differences were noticed in the kinetics of the recovery of single foci in FRAP experiments.

5. Cellular responses to genotoxic stress

Compounds forming covalent DNA adducts are currently included in virtually all cancer therapeutic regimens. However, the way these drugs lead to cell death and preferentially cause cytotoxicity in tumor cells are poorly understood. Annette Larsen (INSERM, Paris, France) investigated the cytotoxic activities of DNA alkylators S23906-1 (an acronycine derivative) [56]

and ET-743 (a tetrahydroisoquinoline alkaloid isolated from a Caribbean sea squirt) [57], two compounds that form bulky adducts influencing the base pairing of adjacent duplex DNA as well as local chromatin architecture. ET-743, in particular, is a very promising drug for which longterm responses were seen in 30% of patients with otherwise chemoresistant soft tissue sarcomas. Mechanistic studies indicated that the cytotoxic activity of these compounds was mediated by the generation of secondary DNA damage following collision with the replication or the transcription machineries and that both primary and secondary DNA lesions were recognized by different DNA repair pathways, which may either increase or attenuate the cytotoxicity. Ongoing studies with these unique compounds aim at the identification of patient subgroups with defined treatment responses as a function of the individual, pathway-specific DNA repair activities.

Bernd Kaina (University of Mainz, Germany) comprehensively summarized today's knowledge on the apoptotic pathways triggered by methylating and chloroethylating agents that are being used in first line therapies of gliomas and malignant melanomas. In the case of methylating agents apoptosis induction requires several key steps in addition to O6-alkylguanine generation, namely replication, mismatch repair and the formation of DSBs. In fact, methylation resistance is inversely correlated to the expression of Msh2, the initial mismatch repair factor. O6-methylguanine-DNA methyltransferase (MGMT) plays a crucial role in the defence against alkylating agents that generate O6-alkylguanine in DNA, a major trigger of genotoxicity and apoptosis. Therefore, screening individual MGMT expression levels in tumors and normal tissues should predict efficacy of methylation-based cancer therapies [58,59]. Similar strategies are conceivable also for treatment of glioblastomas with chloroethylating agents like ACNU, for which Bernd Kaina and his group observed increased cellular sensitivity coupled to DSB accumulation, when p53 is mutated (screening of p53 or HR status).

Frank Grosse (Fritz Lipmann Institute, Jena, Germany) reported on nuclear DNA helicase II (NDH II), also known as RNA helicase A (RHA), which is involved in transcription and RNA processing. He showed that upon transcriptional stress NDH II binds to H2AX phosphorylated by DNA-PK [60]. Interestingly, NDH II itself was phosphorylated by DNA-PK and interacted with the Werner syndrome helicase (WRN), an enzyme associated with premature aging and predisposition to tumorigenesis, which also associates with γ H2AX. Direct protein-protein interactions between WRN and NDH II were mapped to the N-terminal double-strand RNA-binding domain II and C-terminal RGG box of NDH II and the N-terminal exonuclease domain of WRN [61]. WRN inhibited the DNA-dependent NTPase and DNA helicase activities of NDH II, while the exonuclease activity of WRN was increased by the presence of NDH II. These results suggest that NDH II and WRN functionally cooperate, particularly at sites of active transcription. These data are in accordance with the view that transcription stalling by a DNA lesion causes changes in the surrounding chromatin and γ H2AX foci formation. γ H2AX in turn attracts NDH II and WRN to remove the stalled RNA strand from the transcription bubble and to prevent transcription activated recombination events.

Jürgen Thomale (Institut für Zellbiologie, Essen, Germany) demonstrated maturation-dependent alterations in DNA repair function for the lymphohematopoietic system [62]. Because little information is available on the regulatory mechanisms underlying these changes, Jürgen Thomale and co-workers correlated the expression of DNA damage response genes and the functional repair capacity of cells at distinct stages of human hematopoietic differentiation [63]. Comparing fractions of mature lymphocytes (CD34⁻), progenitor cells (CD34⁺ 38⁺) and stem cells (CD34⁺ 38^{low}) from cord blood, they observed stringently regulated differentiation-dependent shifts in both the cellular processing of DNA lesions and the expression profiles of related genes. During blood cell development, the removal of DNA adducts, the resealing of repair gaps and the resistance to DNA-reactive drugs clearly increased in stem cells or mature lymphocytes compared with progenitor cells of the same individual. On the other hand, the vast majority of differentially expressed repair genes was consistently upregulated in the progenitor fraction. Only RAD23 and ATM expression levels positively correlated with repair function, suggesting key regulatory functions. Jürgen Thomale proposed that the organism aims at protecting the small number of precious and slowly dividing stem cells by extensive DNA repair, whereas fast-proliferating progenitor cells, once damaged, are rather eliminated by apoptosis.

This session ended with seven proffered papers. First, Simone Difilippantonio (NIH, Bethesda, USA) from the Nussenzweig lab had made the effort to assess the contribution of distinct regions of Nbs1 to the ATM-dependent DNA damage response *in vivo* by reconstituting Nbs1 knockout mice with Nbs1 mutated within distinct regions: Nbs(657Δ5), corresponding to a hypomorphic mutation found in 95 % of all NBS patients, Nbs(H45A) mutated in the N-terminal FHA domain, Nbs(tr735) deleting the C-terminal ATM binding domain and Nbs(tr645) with neither the ATM nor the Mre11 binding domain. Surprisingly, none of the mutations disrupted ATM binding. Further experiments indicated that the FHA rather than the ATM binding domain is required for chromatin retention, efficient DSB repair and G2/M and S phase checkpoint arrest, whereas the ATM binding (but not FHA) domain contributes to IR-induced apoptosis [64,65]. To investigate the DNA damage-induced responses of DNA topoisomerases, Christian Mielke (Heinrich Heine University, Düsseldorf, Germany) irradiated cells expressing GFP-tagged enzymes with an UVA laser focused through a confocal microscope at confined areas of nuclei. Both topoisomerase II and I localized within seconds to UVA-induced lesions, suggesting that both enzymes function as components of DNA damage recognition and/or cofactors of DNA repair processes. Accumulation of both catalytically active and inactive topoisomerase I versions argues against suicidal complexes and in favor of non-covalent binding to damaged DNA [66]. Then, Walid Rachidi (CEA, Grenoble, France) addressed the question of radiosensitivity of epithelial stem cells of the human epidermis. In the light of Jürgen Thomale's findings, it was of interest that, here, stem cells turned out to be more IR-resistant, whereas progenitor cells were more sensitive. Consistently, stem as compared to progenitor cells performed DSB repair more efficiently as was indicated by the kinetics of γ H2AX foci disappearance. Through the generation of conditional

Atr knockout mice using Nestin-cre, Pierre-Olivier Frappart (St. Jude Children's Research Hospital, Memphis, USA) was able to demonstrate the critical role of Atr in central nervous system development. Mechanistic studies with these mice and animals carrying additional p53 knock-out indicated that replication fork arrest in highly proliferating neural stem and progenitor cells devoid of Atr caused DSB formation (γ H2AX foci) followed by activation of the ATM-p53 pathway, leading to apoptosis. Markus Christmann (University of Mainz, Germany) outlined his results demonstrating that the JNK/c-Fos pathway protects against UV-C light induced cell death via upregulation of XPF and XPG, which stimulates NER [67,68]. Taija Kiviharju-af Hällström (University of Helsinki, Finland) dissected the molecular causes possibly underlying the high incidence and multifocality of prostate cancer. Starting from the observation that clearance of IR-induced γ H2AX foci in human primary prostate epithelial cells (HPECs) is delayed, she identified low level expression of Wee1A tyrosine kinase as the cause of the striking inability of HPECs to control checkpoint arrest. Finally, Jean-Christophe Bourdon (University of Dundee, UK) discussed the complexity of p53 isoforms and their specific impact on transcriptional activation of target genes [69] and breast tumorigenesis. Thus, detailed analysis of 174 primary breast tumors revealed that expression of variant Δ 133p53 β was associated with poor survival and higher numbers of nodes, possibly through an increase in cell motility; p53 γ was associated with good prognosis. These findings stressed the clinical relevance of isoform analysis to better understand biological properties and drug responses related to the p53 status [70].

6. DNA repair machinery within the chromatin context

The histone acetyltransferase (HAT) Tip60 belongs to a multimolecular complex which contains other chromatin modifying enzymes such as p400 (ATPase of the SWI/SNF family) and helicases Tip49a and Tip49b. Didier Trouche (CNRS, University of Toulouse, France) reported that Tip60 not only binds and acetylates p53 within its DNA binding domain but, indeed, is a crucial component of the p53 pathway. More precisely, Tip60 participates in cell cycle arrest following DNA damage by enabling p53 to activate p21 expression [71]. In contrast, p400 represses p21 expression in unstressed cells. Tip60 and p400 have thus opposite effects on p21 expression in the absence of DNA damage. This antagonism was further shown to rely on the inhibition of Tip60 function by p400, a property that is abolished following DNA damage [72]. Concerning the role of Tip60 in DNA repair, Didier Trouche showed that RNAi-mediated silencing caused compromised HR and exacerbated sensitivities to X-rays, IR and MMC. Chromatin immunoprecipitation (ChIP) experiments unveiled that Tip60 associates with I-Sce I-induced DSBs in a manner depending on Rad50, although the Tip60/MRN complex dissociates within 10 min following ATM activation. From the fact that Tip60 shows the features of a tumor suppressor and consistently was found to be underexpressed in colon cancer biopsies, Didier Trouche and colleagues are currently aiming at strategies for the development of drugs targeting the inhibition of Tip60 by p400.

Inheritance and maintenance of the DNA sequence (genetic information) and its organization into chromatin (epigenetic information) are both central for eukaryotic life. In particular, orchestrating chromatin unfolding and restructuring upon drastic processes such as DNA replication or repair is a real challenge [73,74]. Geneviève Almouzni (Institut Curie-CNRS, Paris, France) and co-workers have isolated several factors that participate in chromatin assembly, including chromatin assembly factor-1 (CAF-1), histone regulator A (HIRA) and anti-silencing function 1 (Asf1) [73,74]. HIRA is associated with the H3.3-H4 complex and is critical for a DNA-synthesis-independent nucleosome assembly process [75]. CAF-1 is an evolutionarily conserved three-subunit protein found in a specific pre-deposition complex containing the major S phase histones H3.1 and H4 [76], consistent with its unique ability to preferentially deposit newly synthesized H3-H4 onto replicating DNA. CAF-1-mediated histone deposition is aided by Asf1, a highly conserved histone H3.1-H4 chaperone and thereby controls the flow of newly synthesized S phase histones, which becomes particularly important during recovery from replicational stress [77]. Most recently, Geneviève Almouzni started to integrate the functions of these factors during DNA repair *in vivo*. Thus, she showed that new histone H3.1 incorporation at UV damage sites follows NER via a CAF-1 dependent pathway, in this case, however, outside S phase [78].

This session ended with four proffered papers. First, Giuseppina Giglia-Mari (Erasmus MC, Rotterdam, The Netherlands) questioned approaches involving cultured cells (under constant stress and in a proliferative state) and their extrapolation to somatic tissues (mostly differentiated and post-mitotic cells). By creating a knock-in mouse model expressing fluorescently tagged XPB under the control of the endogenous promoter, Giuseppina Giglia-Mari and her colleagues were able to analyse TFIIH dynamics in living tissues. Their observation of a surprisingly low transcription-dependent mobility of TFIIH in highly differentiated cells as compared to cultured cells highlights the need for a revision of current models mostly based on analyses of cultured cells. Then, Franziska Knauf (Gesellschaft für Schwerionenforschung, Darmstadt, Germany) addressed chromatin modifications in response to DNA damage by using various irradiation conditions (heavy ions, carbon ions, X-rays). Notably, she showed that using heavy ions Tip60 foci formed along ion tracks, generally colocalizing with γ H2AX foci in an ATM-modulated manner. Pursuing on chromatin dynamics with a more mechanistic focus, Christophe Lavelle (CNRS, Villejuif, France) stressed the fact that DNA in the nucleus is almost never naked, raising the question of how various repair and transcription enzymes manage to overcome the nucleosome barrier [79]. Namely, he showed that, far from being merely “tuna cans” sticking to DNA, nucleosomes are highly polymorphic and dynamic entities [80,81]. Using atomic force and electron microscopies, Christophe Lavelle and colleagues presented evidence of chromatin remodeling occurring through Rad51 nucleoprotein filament formation, thus defining an additional potential role for recombinases along with a new kind of remodeling mechanism. Lastly, Thomas Schleker (Friedrich Miescher Institute, Basel, Switzerland) showed that phosphorylation of H2A, the H2AX counterpart in yeast, is not only a signal for the DNA

repair machinery but occurs also at replicating rDNA and naturally elongating telomeres, suggesting its influence in nuclear organisation besides its well-acknowledged role in DNA damage signaling.

7. Genotoxicology and testing

Daniel Marzin (Institut Pasteur, Lille, France) discussed the pros and cons of DNA repair-based systems in genotoxicity testing. Several tests were developed in bacteria (Rec assay in *Bacillus subtilis* and SOS Chromotest in *Escherichia coli*) but difficulties in metabolically activating the compounds and the production of false positive results limited the use of these assays. Importantly, the latter was recently recognized as the major pitfall of today's genotoxicity testing approaches [82]. Among the assays in mammalian cells, UDS (unscheduled DNA synthesis), has a relatively low sensitivity. With the development of the comet assay, a more sensitive assay became available [83]. The comet assay additionally identifies clastogens and oxidative and alkylation DNA damage via inclusion of Fpg or Ogg1, which, however, bears technical problems. Daniel Marzin concluded that these and additional drawbacks like the ubiquitous use of repair defective cell lines (e.g. p53 dysfunctional CHO-K1, CHL, V79) explain, why so far DNA repair has not become the preferred endpoint of genotoxicity testing as compared to mutations.

Over the last years Andrea Hartwig (Institute of Food Technology and Food Chemistry, Berlin, Germany) and co-workers have provided major insight into the mechanisms of metal-induced genotoxicities [84]. Regarding this class of compounds quantification of DNA repair activities revealed that impaired rather than elevated DNA repair activities were observed after treatment. Mechanistic studies, then, showed that these compounds frequently target zinc finger domain DNA repair proteins such as XPA, p53 and PARP. For PARP in specific, it was shown that different metal compounds inactivate the enzyme via different molecular mechanisms ranging from replacement to release of Zn. Use of corresponding PARP activity tests, thus, have the potential to represent novel biomarkers for the assessment of metal compound genotoxicities.

This last session ended with two proffered papers. First, Anne-Laure Raffin (CEA, Grenoble, France) and colleagues used an *in vitro* DNA repair assay on microsupport [85] to simultaneously study the repair of a variety of immobilized DNA lesions in nuclear extracts. Indeed, extracts from XP patients showed delayed repair, with a particularly strong decrease in the activity of XPC extracts for all lesions tested. Lastly, Yannick Saintigny (CEA, Fontenay aux Roses, France) evaluated the effect of sub-lethal doses of contamination by 3H- or 14C-thymidine on cell survival, DSB formation, cell cycle regulation, mutagenesis and HR. The results showed mutagenesis already at very low doses of incorporation, a dramatic increase of HR at higher doses, DSB formation and G2/M arrest. He emphasized the fact that the remarkable survival of contaminated cells carrying genetic alterations may increase the risk of germline transmissions of genetic alterations and the risk of cancer.

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