thesis, (ii) the maintenance of the proper ratio of dNTP components, and (iii) the elimination of the modified nucleotides from the pool. Perturbed regulation results in the appearance of DNA lesions compromising genome integrity mostly by base excision (BER) or mismatch repair. Uracil bases are frequent lesions that can occur in DNA. dUTPase has the essential role to prevent dUMP incorporation into DNA, and an uracil DNA glycosylase, UNG, a component of BER is responsible for uracil removal from the genome.

From previous observations we learned that the fruit fly lacks UNG from the three components and possess only a statio-temporal expression of dUTPase. We showed that the uracil content of DNA depends only on dUTPase expression in this organism. I also examined the transcriptional regulatory mechanism that forms dUTPase expression pattern. My results suggest that DRE motifs located in dUTPase promoter may be responsible for a cell cycle dependent expression, but there might be a DRE independent gonad-specific regulation as well. We also showed that compromising dUTPase expression in proliferating tissues causes cell autonomous tissue degradation during pupal stages. Furthermore, we showed genomic uracil accumulation, DNA fragmentation and DNA damage response activation these tissues.

SW01.S3–20
Application of repair enzymes to improve the quality of the DNA template in PCR amplification of degraded DNA

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Despite the fact that DNA in living organisms is used as the main carrier of genetic information, the chemical stability of this molecule is limited. In living organisms, such DNA damage can lead eventually to the emergence of mutations, tumors, and contribute to aging. During the life of the organism, the repair systems resist the accumulation of damage in DNA, but with the death of the organism, these processes cease working, and the accumulation of DNA damage becomes irreversible. The accumulation of damage in DNA can be a problem when it is necessary to analyze its sequence. For example, the efficiency of PCR is sharply reduced if the template is subjected to oxidation or apurinization. This is particularly actual in studies of 'ancient DNA' and DNA in the forensic practice. We are developing a system in which repair enzymes are used to improve the quality of degraded DNA matrices before PCR.

SW01.S3–21
Characterization of the *in vivo* functions of PrimPol, a novel TLS primase-polymerase

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Genomic integrity depends critically on the fidelity and efficiency of DNA replication. The catalytic site of the replicative DNA polymerases is compact and intolerant of DNA lesions such are thymine dimers induced by UV light. As a consequence, translesion DNA synthesis (TLS) is is used to bypass such lesions and facilitate replication fork progression. We have identified PrimPol, a yet uncharacterized protein encoding a primase domain of the AEP (Archaeo-Eukaryotic Primase) family. *In vitro*, PrimPol is able to bypasses oxidative 8-oxo-G and UV lesions in DNA, suggesting a role in TLS. PrimPol localizes to both the nucleus and mitochondria indicating it may play important roles in genome maintenance in both compartments. In order to investigate the role of PrimPol *in vivo*, we have generated mice lacking its expression. PrimPol−/− mice exhibit increased genomic instability that is exacerbated upon UV damage or aphidicolin treatment. Consistent with this, damaged cells show sustained phosphorylation of Chk1 and p53 in the absence of PrimPol. Additionally, the expression of PrimPol is highest in UV sensitive tissues including the eye and skin. To validate the *in vivo* function of PrimPol we analyzed histological changes in the skin of mice upon acute UV exposure. UV damaged skin in PrimPol−/− mice showed marked epidermal hyperplasia compared to wild type. Xeroderma pigmentosum (XP) is a disease caused by the mutation of the TLS polymerase Pol η, responsible for the repair of TT dimers. Depletion of Pol η in PrimPol−/− MEFs leads to synergistic sensitivity to UV damage. We have also observed increasesd mitochondrial DNA copy number in PrimPol−/− mice in tissues with high metabolic turnover implying that loss of PrimPol may impair the repair of oxidative damage leading to mitochondrial stress. Based on these findings, we hypothesize that PrimPol functions in TLS by bypassing TT dimers after UV irradiation and functions in parallel to Pol η. This activity is likely required for the maintenance of both the mitochondrial and nuclear genomes in response to UV and oxidative base damage. PrimPol knockout mice provide a model system for understanding the potential role of this enzyme in the etiology of sunlight induced skin cancers and premature ageing induced by oxidative stress. Current results from the ongoing characterization of these animals will be presented.

SW01.S3–22
dUTPase based switch controls transfer of virulence genes in order to preserve integrity of the transferred mobile genetic elements

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dUTPases ubiquitously regulate cellular dUTP levels to preserve genome integrity. Recently, several other cellular processes were reported to be controlled by dUTPases including the horizontal transfer of *Staphylococcus aureus* pathogenicity islands (SaPI). SaPIs are mobil genetic elements that encode virulence enhancing factors e.g. toxins. Here, phage dUTPases were proposed to counteract the repressor protein (Stl) and promote SaPI excision and transfer. A G protein-like mechanism was proposed which is unexpected in light of the kinetic mechanism of dUTPase.

Here we investigate the molecular mechanism of SaPI transfer regulation, using numerous dUTPase variants and a wide range of *in vitro* methods (steady-state and transient kinetics, VIS and fluorescence spectroscopy, EMSA, quartz crystal microbalance, X-ray crystallography).

Our results unambiguously show that Stl inhibits the enzymatic activity of dUTPase in the nM concentration range and dUTP strongly inhibits the dUTPase: Stl complexation. These results identify Stl as a highly potent dUTPase inhibitor protein and disprove the G protein-like mechanism. Importantly, our results clearly show that the dUTPase:dUTP complex is inaccessible to the Stl repressor. Unlike in small GTPases, hydrolysis of the substrate nucleoside triphosphate (dUTP in this case) is required prior to the interaction with the partner (Stl repressor in this case). We propose that dUTPase can efficiently interact with Stl and induce SaPI excision only if the cellular dUTP level is

low (i.e. when dUTPase resides mainly in the apo enzyme form) while high dUTP levels would inhibit SaPI transfer. This mechanism may serve the preservation of the integrity of the transferred SaPI genes and links the well-known metabolic role of dUTPases to their newly revealed regulatory function in spread of virulence factors.

SW01.S3–23
Zinc finger nucleases generate DNA double strand brakes and modification in *Chlamydomonas reinhardtii*

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The green microalga *Chlamydomonas reinhardtii* is a valuable model organism for the study of fundamental biological processes such as photosynthesis, metabolism, phototaxis, flagella assembly, circadian rhythmicity, the cell cycle and mating. Important, *Chlamydomonas* preserves characteristics of plant and animal cells and is regarded as their common ancestor. Similarity with mammalian cells makes this alga a voluble model for human ciliopathies that result from defects in primary cilia. The computer analysis of *Chlamydomonas* genome databases has revealed the central homologous recombination (HR) protein RAD51 and its paralogs which are characteristic of higher eukaryotes. But yet, homologous recombination (HR) and DNA non-homologous end joining (NHEJ) function in *Chlamydomonas* are still poorly understood. Similar as in higher eukaryotes, when exogenous DNA is introduced into *Chlamydomonas* cells, HR is three orders of magnitude less efficient than NHEJ that makes site-specific mutagenesis through gene targeting difficult. For site-specific genomic modification and characterization of HR and NHEJ products we designed, constructed and used zinc finger nucleases (ZFNs). We demonstrated by targeted disruption of the *CHRI* gene (encoding channelrhodopsin-1) that in *Chlamydomonas* modular assembly-based ZFNs can introduce site-specific double stranded breaks (DSB) and stimulate both NHEJ and HR with the supplied template DNA. We found that about 1% of the clones expressing active *CHRI*-specific ZFN contained a mutated *CHRI* locus. If modified template DNA was supplied, *CHRI* alterations were exactly copied through a pathway best described by the synthesis-dependent strand annealing mechanism. Without template DNA DSB repair went through mutagenic NHEJ. Mutations generated through this pathway included long insertions copied from the chromosome or deletions between short repeats. Other experiments on analysis of mutations generated by earlier described ZFNs Zif268 or GZ1/GZ3 confirmed that the most frequent mutations were deletions from 8 until longer than 400 bp which took place between repeated sequences from 2 (GC) unlil 8 bp (GGTCGGCC). Our data suggest that in *Chlamydomonas* an alternative microhomology-mediated end-joining pathway is high efficient: this mechanism is initiated by ssDNA resection, mediated by base pairing between microhomologous sequences and Ku-independent. For further study of DSB repair and the gene targeting machinery in *Chlamydomonas*, ZFNs specific to proteins of HR and NHEJ pathways will be designed and validated for site-specific mutagenesis.

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SW01.S3–24
Comparative analysis of interaction of PARP1 and PARP2 with apurinic/apyrimidinic DNA

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One of the most frequently formed DNA damages (about 10 000 lesions per mammalian cell per day) is apurinic/apyrimidinic (AP) site. Unrepaired, AP sites present mutagenic and cytotoxic consequences to the cell. UV-light or X-ray irradiation dramatically increase the number of AP sites. AP sites in mammalian cells are generally processed via functioning of base excision repair (BER) system. One of key regulatory proteins in BER is PARP1. Its role in response to DNA damage has been widely illustrated, the contribution of another DNA-dependent PARP, PARP2, has not been studied in detail. It is the closest homolog of PARP1.

In this work we studied interaction of PARP2, with AP sites and several BER proteins taking part in the AP sites processing. We demonstrated the capability of PARP2 to interact with abasic sites forming stable complexes PARP2-abasic DNA. The repair of AP sites in canonical BER is initiated through hydrolysis by AP endonuclease 1 (APE1) or β-elimination via the activity of DNA glycosylases and other enzymes with associated AP lyase activity. PARP1 possesses 5′-dRP/AP lyase activity. In contrast, PARP2 is unable to cleave abasic sites, but like PARP1, it displays 5′-dRP activity. PARP1 and PARP2, although to a lesser extent, are able to inhibit the APE1 activity, with inhibition being regulated by poly(ADP-rybosyl)ation. Like PARP1, PARP2 interferes with the activity of downstream BER enzymes – DNA polymerase β and flap endonuclease 1.

Taken together, our obtained results testify to probable additional regulation of BER by PARP2. Study of the mechanisms of PARPs involvement in BER can be essential for developing new drugs for selective inhibition of these proteins.

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SW01.S3–25
RecA730 dependent suppression of DNA repair deficiency in RecA loading mutants of *Escherichia coli*

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Homologous recombination is essential for DNA repair and the maintenance of genome integrity. The most dangerous DNA damage are double-strand breaks (DSBs) which can arise during normal cell cycle or as a consequence of the action of DNA damaging agents [1]. In *Escherichia coli*, repair of DSBs occurs by homologous recombination. The central part of recombination process is binding of RecA protein to ssDNA, i.e. production of RecA filament. DSBs can be processed into a RecA filament by the action of three enzymatic activities: helicase, 5′–3′exonuclease and RecA loading onto ssDNA. These activities are provided by the RecBCD enzyme in wild type cells or by the RecF pathway gene products in the *recBC sbcBC(D)* cells [2].

The RecA730 mutant protein binds to ssDNA more efficiently than SSB protein, and consequently it is able to produce a RecA filament without the help of RecFOR mediators [3]. We wanted to test whether the *recA730* mutation can suppress DNA repair defi-