that plays a crucial role in this process. Upon DNA damage, the catalytic subunit of replicative polymerase delta, gets degraded in a mechanism which is dependent on the above mentioned protein, which in turn paves the way for translesion polymerase to take over DNA synthesis and carry out lesion bypass.

SW01.S3–17
Novel small molecules that selectively induce transcriptional activity and modulate marks on chromatin

I. Charapitsa and G. Reid
Institute of Molecular Biology, Mainz, Germany

DNA methylation is an important epigenetic regulator of transcription activity, and plays a significant role in development, cell differentiation and tumourogenesis. While mechanisms of *de novo* and maintenance methylation are well studied, active demethylation remains poorly explained. In order to gain a better understanding of this process, we have carried out a chemogenetic screen aiming to find novel small molecules that can reactivate transcription from methylated plasmid DNA. It has resulted in identification of two different chemical series out of the screened 80 000 compounds that restored the expression of the methylated plasmid to unmethylated levels. The screen provided us with a model to study active DNA demethylation. A kinetic, genome wide, massively parallel sequence analysis of GST-MBD pull down gDNA from HeLa cells treated for 24 h with the small molecule IMBMZ-744363 has identified over 2700 regions (138 lie within promoters) as significantly demethylated, comparing to a mock control. Remarkably, one-third of the identified regions contain one or more CREB_EGR1 modules. RNA-seq and pathway analysis showed the involvement of transcription factors EGR1 and CREB1 in the response HeLa cells make to the compound, as well as the activation of kinase cascades. A detailed analysis of EGR1 promoter revealed the responsive element between the first 50 nucleotides prior to the transcriptional start site of EGR1. Subsequent work has demonstrated that a CREB responsive element (CRE) on the minus strand is required, but not sufficient, for the response to the compound. Importantly, six CpG dinucleotides are present in the sequence of the minimal response element, including one within the CRE; mutation of any CpG results in loss of inducibility. We furthermore confirmed the role of CREB in the induction of EGR1 expression by transfecting a dominant negative version of CREB into HeLa cells, which blocked the effect of IMBMZ-744363. As CREB is regulated by phosphorylation, we characterized the phosphorylation status of ser133 of CREB, which becomes transiently phosphorylated in response to IMBMZ-744363. Next, we have also shown activation of kinase cascades signaling to chromatin in response to IMBMZ-744363. Inhibition of PI3Kinase activity, as well as of aurora A kinase, prevents compound induced upregulation of the EGR1 promoter. We also demonstrate that Akt, a downstream target of PI3Kinase, is involved in the response cells make to IMBMZ-744363. Moreover, we have carried out a western blot analysis of few selected active and repressive histone marks and showed significant changes in their total amount: active marks (H3K4me3, H3K35me3, H3K14ac and panH3ac) are increased within the first hours after IMBMZ-744363 treatment, followed by the rise of the repressive ones (H3K27me3) later.

Understanding how small molecules can change epigenetic status of a cell will contribute to our understanding of the role of DNA demethylation in regulating gene expression, which can then be applied in the epigenetic treatment of cancer therapy, as in changing the cell fate, either by inducing pluripotency in adult cells or promoting differentiation in stem cell populations.

SW01.S3–18
Nucleolin overexpression leads to increased quantity of DNA double strand breaks after etoposide treatment on HeLa cells

M. A. Rubtsov[1,2], L. V. Ageeva[1,2], S. V. Razin[1,2] and O. V. Iarovaia[1,2]
[1]*Lomonosov Moscow State University,* [2]*Institute of Gene Biology, Russian Academy of Sciences, Moscow, Russia*

The use of DNA topoisomerase II inhibitors (topoisomerase poisons) in anticancer chemotherapy does not infrequently leads to development of the so-called 'treatment-related' or 'secondary' leukaemias associated with chromosomal translocation t(8,21)(q22;q22) affecting AML1 and ETO genes. Under the action of topoisomerase poisons DNA double strand breaks are formed in the nuclei which can lead to further chromosomal rearrangement. Obviously this requires direct 'contact' of DNA break's flanks of different chromosomes in the same nuclear compartment where damaged DNA repair should occur. Previously we have shown that treatment of Jurkat cells with DNA topoisomerase II inhibitor etoposide leads to increase of the probability of localization of ETO gene on the surface of the nucleoli as well as enrichment of major nucleolar protein nucleolin within ETO containing chromatin locus. We hypothesized that nucleolar proteins may be involved in the DNA double strand breaks repair and that surface of the nucleoli is the compartment where chromosomal translocations might occur.

The aim of this work was to determine the possible role of nucleolin in the processes following treatment of cells with etoposide. By means of transient transfection we have obtained HeLa cells with overexpression of nucleolin. DNA double strand breaks were detected by using of neutral 'comet-assay' and pulsed-field gel electrophoresis.

Analysis of the average molecular weight of DNA fragments formed after cell treatment with etoposide shown that the fragmentation of DNA in cells with nucleolin overexpression used to be higher than in control. Therefore overexpression of nucleolin leads to increased sensitivity of HeLa cells to etoposide treatment. And since we know that nucleolin have the activity of histone chaperone and might be involved in chromatin remodeling we can assume that its overexpression can lead to a general or local chromatin decondensation and consequently increases the number of sites on the DNA available for binding and cleavage by DNA topoisomerase II. Further information about the possible functions of nucleolar proteins in the formation and repair of DNA double strand breaks can be useful for understanding the mechanisms of chromosomal rearrangements, as well as to find new treatments for oncological diseases.

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SW01.S3–19
The impact of dUTPase expression on genome integrity

A. Horvath[1], J. Batki[1], V. Muha[1], G. Rona[1], P. Vilmos[2], M. Erdelyi[2] and B. G. Vertessy[1]
[1]*Institute of Enzymology, RCNS, Hungarian Academy of Sciences,* [2]*Institute of Genetics, Biological Research Centre, Hungarian Academy of Sciences, Hungary*

Accurate and stable DNA synthesis requires the precise regulation of dNTP pool. This regulation involves (i) meeting the requirements of cell cycle or DNA repair dependent DNA syn-

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

A. Dovgerd and D. Zharkov
Institute of Chemical Biology and Fundamental Medicine, Siberian Branch of the Russian Academy of Sciences, Novosibirsk, Russia

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

I. Stevanovic[1], L. Blanco[2], A. Doherty[3] and T. Stracker[1]
[1]*IRB Barcelona, Oncology Programme, Barcelona, Spain,* [2]*Centro de Biologia Molecular, CSIC, Madrid, Spain,* [3]*Genome Damage and Stability Center, University of Sussex, UK*

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

J. E. Szabo[1], V. Nemeth-Pongracz[1], V. Papp-Kadar[1], K. Nyiri[1], B. Besztercei[1], K. Liliom[1], G. Rona[1], H. Palinkas[1], I. Zagyva[1,2], I. Leveles[1,2], A. Bendes[1], J. Toth[1] and B. G. Vertessy[1,2]
[1]*Institute od Enzimology, RCNS, HAS,* [2]*Department of Applied Biotechnology and Food Sciences, University of Technology and Economics, Budapest, Hungary*

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