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Orals

O01

The role of helicase-like transcription factor in nucleotide excision repair-mediated removal of excised oligonucleotides

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Nucleotide excision repair (NER) is a versatile DNA repair mechanism that protects the genome against a broad spectrum of helix-distorting DNA damage, including UV-induced DNA lesions. NER deficiency can lead to the development of cancer and/or accelerated ageing, underscoring the importance of the pathway in human physiology. In the past decades, many crucial insights have been obtained as to how NER recognizes damaged DNA by XPC or by stalling of Pol II and how TFIIH verifies the lesion and subsequently unwinds the DNA lesion in such a way that the DNA surrounding the lesion can be incised by the XPF/ERCC1 and XPG endonucleases. Even though it is an essential step for efficient restoration of the single-stranded gap by DNA synthesis, thus far it remains largely unknown how the incised, damage-containing oligonucleotide is removed from the chromatin. To identify factors involved in the eviction of damage-containing oligonucleotides, we performed quantitative interaction proteomics using a cellular model in which a YFP-tagged version of the largest TFIIH subunit, XPB, is expressed at endogenous levels. Interestingly, our results identified the helicase-like transcription factor (HLTF), a SWI/SNF family chromatin remodeler that also possesses DNA helicase and ubiquitin ligase activities, as a TFIIH interactor. This UV-induced interaction is XPC and XPG dependent, suggesting that HLTF is recruited to TFIIH following incision. Using live cell imaging, we show that HLTF knockdown results in prolonged chromatin binding following DNA damage of TFIIH, XPG and XPF. Despite the presence of the complete incision complex upon HLTF depletion, we observed a reduced number of excised damage-containing oligonucleotides in the nucleoplasm, suggesting that HLTF might play a role in the efficient release of incised, damage-containing oligonucleotides. Efficient release of these oligonucleotides is expected to be essential for damage-induced DNA synthesis. In line with this, following HLTF depletion, PCNA recruitment to sites of DNA damage and gap-filling DNA synthesis (UDS) are strongly reduced. Together, our results provide evidence that HLTF is required for proper progression through the NER reaction by stimulating the release of damage-containing oligonucleotides from chromatin, which most likely represent an important step for regulating the timely initiation of gap filling and DNA damage signalling.

O02

Novel Mediator function connecting transcription and nucleotide excision repair

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Transcription and maintenance of genome integrity are fundamental and extremely complex functions of the cell. Deregulation of transcription and defects in DNA repair lead to a number of serious pathologies. How transcription and DNA repair are coordinated *in vivo* remains one of the key biological questions. Mediator is a very conserved multiprotein complex that plays a crucial role in transcription regulation in all eukaryotes. Recently, we discovered a novel Mediator function as a link between transcription and DNA repair via direct contact with Rad2 endonuclease, the yeast homolog of human XPG protein involved in nucleotide excision repair (NER). We suggest that Mediator is involved in transcription-coupled repair by facilitating Rad2/XPG recruitment to transcribed genes. Our recent results indicate that the link between Mediator and DNA repair can be also extended to other NER proteins. Addressing the conservation of the mechanisms in human cells, we showed that Mediator interacts with XPG protein and that Mediator chromatin binding correlates with that of XPG. We analysed a potential implication of the Mediator link to NER in xeroderma pigmentosum/Cockayne syndrome. Pol II is the first component in transcription-coupled repair that encounters the DNA damage. Both Mediator and Rad2/XPG interact with Pol II. It remains to be determined how all these components work together. Using genetic and genomic approaches, we demonstrated that Rad2 shuttles between Mediator on regulatory regions and Pol II on transcribed regions. Our data provide mechanistic insights into the functional interplay between Mediator, Rad2 and Pol II related to transcription-coupled repair. They also suggest that the functions of Mediator in transcription and DNA repair are closely related. In conclusion, we propose Mediator as a new player in DNA repair mechanisms coupled with transcription activation. Our work thus contributes to new concepts of the functional interplay between transcription and DNA repai,r

and might give insights into our understanding of human diseases.

O03
Elucidating the role of active transcription in keeping genome integrity in check

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Complex molecular responses preserve gene expression accuracy and genome integrity in the face of environmental perturbations. We have recently shown that in response to UV irradiation, synchronous and dynamic release of elongating RNA polymerase II (RNAPII) 'waves', created by promoter–proximal pause release, efficiently scan all actively transcribed protein-coding genes and long noncoding RNAs. This mechanism maximizes and accelerates the recognition of DNA lesions and therefore enhances transcription-coupled nucleotide excision repair (TC-NER). Although initiation of transcription should be the 'feeding source' of this process, the levels of pre-initiating RNAPII have been reported by us and others to be significantly reduced. By contrast, recent studies have suggested that transcription initiation is not as profoundly affected by UV as transcription elongation. To address this paradox and elucidate the dynamics of transcription initiation upon UV stress, we functionally dissected the reorganization of the transcriptional landscape. Our data revealed a significant gain in chromatin accessibility (measured by ATAC-seq and ChIP-seq of active and silent histone marks) at active promoters and enhancers, which was fully correlated to increased transcription elongation and NER activity. In line with this, we demonstrated that transcription initiation is not inhibited and it remains active at these loci, in spite of unnoticeable levels of preinitiating RNAPII. Indeed, by applying transcription elongation and initiation inhibitors during the early stress-recovery phase, we found that RNAPII molecules are normally assembled at pre-initiation complexes but are instantaneously phosphorylated and proceed into elongation. The fact that initiation events occur rapidly and continuously explains how cells guarantee a steady influx of RNAPII from transcription start sites (TSSs) to promote uniform and accelerated surveillance of the whole transcribed genome, including the regulatory regions encoded at enhancer RNAs (eRNAs) and PROMoter uPstream Transcripts (PROMPTs). The resulting increased damage-sensing activity by RNAPII boosts DNA damage removal by TC-NER at vital sequences regardless of damage location and transcript stability or expression level. In accordance with these findings, we uncovered low and homogenous rates of mutational signatures associated with UV irradiation or cigarette smoke across all active transcribed regions in exposed cancer tissues such as melanoma and lung adenocarcinoma. Our study provides unanticipated insights into how active transcription maintains healthy genomes and how, if defective, it may contribute to health-impairing disorders.

O04
Cockayne syndrome B links the polymerase-associated factor 1 complex to RNA polymerase II to restart transcription after DNA damage repair

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The coordinated transcription of genes involves RNA polymerase II (RNAPII) enzymes, which pull DNA through their active sites. DNA lesions block RNAPII progression along the DNA and cause major complications during transcription. Persistent DNA lesions in transcribed strands induce transcriptional arrest and can trigger apoptosis. The transcription-coupled nucleotide excision repair (TCR) pathway ensures the efficient removal of transcription-blocking DNA lesions from transcribed strands. The TCR-mediated clearing of DNA lesions is essential but not sufficient for the restart of transcription following DNA repair, suggesting the involvement of unknown mechanisms that regulate restart. Through proteomics screens, we identified the polymerase-associated factor 1 complex (PAF1C) as a strong DNA damage-specific interactor of the Cockayne syndrome B (CSB) protein. Moreover, we found that PAF1C strongly associates with RNAPII in response to DNA damage in a manner strictly dependent on the TCR-specific CSB protein. Visualization of nascent transcripts by 5-ethynyl-uridine labelling revealed that knockdown of PAF1 impairs transcription restart after exposure to UV light, which could be rescued by re-expression of PAF1. However, specifically measuring TCR-associated unscheduled DNA synthesis (TCR-UDS) revealed that PAF1 is dispensable for the repair of UV-induced lesions in transcribed strands. These findings reveal a dedicated CSB–PAF1 axis that is specifically required to restart transcription after the repair of transcription-blocking DNA damage has been completed. To address where PAF1-dependent transcription restart takes place in the genome, we are currently mapping PAF1-binding sites in response to DNA damage in a genome-wide fashion. These findings provide a mechanism for transcription restart following DNA damage repair in human cells.

O05
Clinical and genetic investigation of *Xeroderma pigmentosum* in Tunisia: current situation and perspectives

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Xeroderma pigmentosum (XP) is one of the most severe genodermatoses and is characterized by an extreme sensitivity to UV rays and hence a high risk of skin cancers. XP is rare worldwide,

with a prevalence of 1 : 1 000 000 in the USA and Europe and 1 : 100 000 in Japan. However, its seems to be relatively frequent in Tunisia (1 : 10 000), which has been broadly explained by the high level of consanguinity in the country. Over the past 12 years, clinical and genealogical data have been collected for 205 families that include more than 300 patients with XP, the largest cohort of XP ever reported. Molecular investigations allowed the identification of seven complementation groups (XP-A, XP-C, XP-D, XP-E, XP-F, XP-G and XP-V) being responsible for 25%, 40%, 10%, 5%, 1%, 1% and 15% of cases, respectively. The remaining patients, who represent only 3% of the total, have a heterogeneous group of UV-sensitive syndromes or XP-like phenotypes. Our findings showed the presence of several founder mutations with specific geographical distributions, mainly p.V548Afs*25 in the *XPC* gene, p.Arg228* in *XPA* and g.36847 40771del3925 in *POLH*, which are shared among other Maghrebian patients. Additional founder mutations specific to the Tunisian population have been also identified. Many private mutations have been also found. Genetic diagnosis of XP is performed for Tunisian, Libyan and Algerian patients following a three-step strategy (i) targeted founder mutation screening, (ii) targeted gene sequencing and (iii) whole exome sequencing. In the absence of an adequate treatment for this life-threatening disease, understanding its molecular basis has paved the way for genetic counselling and prenatal diagnosis, which have been performed for many at-risk families (15 XP-A and 30 XP-C). Identification of causative mutations has allowed the implementation of cascade screening among affected families on the one hand and prepared the way for a national newborn screening programme on the other. Patient monitoring illustrates the importance of genetic diagnosis for better health care of patients with XP. Indeed, with the help of the patient support group and the multidisciplinary staff (dermatologists, ophthalmologists, neurologists, psychologists and geneticists), we have noticed a considerable raising of awareness not only in the medical and scientific community but also among the public and most importantly the patients' families. This is well illustrated by the improvement in disease prognosis and the increasing number of couples seeking prenatal diagnosis. Moreover, we have discovered ultra-rare forms of photogenodermatoses related to XP mutations in patients with no personal history of skin cancers.

O06
New disease genes for trichothiodystrophy

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Trichothiodystrophy (TTD) is a rare hereditary disorder in which typical hair alterations are associated with skin, neurological and growth abnormalities. About half of patients with TTD show hypersensitivity to UV radiation resulting from defects in nucleotide excision repair (NER). These photosensitive cases are caused by mutations in any of three genes encoding distinct subunits of the TFIIH complex that operates both in NER and transcription. A scenario of extensive heterogeneity with many genes, each involved in a limited number of cases, is instead emerging for the NER-proficient or nonphotosensitive form of TTD. Mutations in the uncharacterized *MPLKIP/TTDN1* gene account for less than 20% of the nonphotosensitive TTD cases, whereas a single family showing X-linked TTD has been found mutated in *RNF113A*, whose product appears to participate in the spliceosome and in signalling of alkylation damage. Recently, we found that mutations in the *GTF2E2* gene are responsible for the TTD clinical outcome in few unrelated cases. *GTF2E2* encodes the beta subunit of TFIIE, the basal transcription factor that interacts with TFIIH and sustains its function in transcription. Now, by using next-generation sequencing approaches in cells from still unsolved cases, we have identified pathogenic variants in two additional genes that encode strictly related factors participating in protein synthesis. Functional alterations resulting from the identified variants will be presented. The expanding spectrum of TTD-associated genes further increases the complexity of TTD aetiopathogenesis. In addition to impairments in genome maintenance and transcription failure, anomalies in translation also appear to contribute to TTD clinical features.

O07
Adeno-associated virus-mediated gene delivery to treat Xpg$^{-/-}$ mice

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Cockayne syndrome (CS) is a rare disease characterized by neurodegeneration and premature ageing. CS is caused by mutations in various genes involved in DNA repair mechanisms. One subtype of CS is caused by mutations in the xeroderma pigmentosum group G (XPG) protein, an endonuclease encoded by the *ERCC5* gene. The hybrid C57BL6/FVB F1 Xpg$^{-/-}$ mouse model developed by Drs. Hoeijmakers and Vermeij replicates the CS phenotype well. The overall aims of this project are to: (i) determine whether adeno-associated virus (AAV)-mediated gene therapy can prevent development of the CS phenotype in these mice through neonatal administration and (ii) determine to what extent AAV gene therapy can halt or reverse progression of the disease process following adult administration. To do this, we are evaluating the impact of gene therapy on the hybrid Xpg$^{-/-}$ knockout mouse model following injections of AAV9-ERCC5 using several doses at one of two alternative time-points (neonates or adults). Untreated Xpg$^{-/-}$, AAV treated Xpg$^{-/-}$ and WT mice are

evaluated weekly by neurological, physical and behavioural examinations for up to 24 weeks. Mice are euthanized upon reaching a moribund state or at 24 weeks and tissues are harvested for further analyses. Thus far, *ERCC5* gene expression has been observed in all AAV-treated groups, and the highest-dose neonatal treatment cohort has displayed improvements in several functional domains including survival. Further analyses are in progress to evaluate the full potential of gene therapy for CS.

Oo8
Endogenous formaldehyde genotoxicity reveals Cockayne syndrome in mice

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Cockayne sdyndrome (CS) is a rare disorder with complex pathology, characterized by a failure to thrive, premature ageing and progressive neurological defects. Despite this heterogeneity, CS occurs due to mutations in just one of two genes, *CSA* and *CSB*, known to play a role in transcription-coupled nucleotide excision repair (TC-NER). Paradoxically, *Csb* knockout mice do not phenocopy humans. Moreover, while the typical DNA damaging agent used to study TC-NER is UV, this is unlikely to be the endogenous source of DNA damage driving CS, due to the nature of the clinical features. Recently, our laboratory identified that reactive aldehydes such as formaldehyde can act as endogenous sources of DNA damage, and we put forward a two-tier protection mechanism against this aldehyde. Tier 1 consists of the enzyme ADH5n which detoxifies formaldehyde and Tier 2 consists of DNA crosslink repair, which removes crosslinks caused by formaldehyde. We now report that when we create mice that lack *Adh5* and *Csb*, we observe severely growth-retarded $Adh5^{-/-}Csb^{-/-}$ mice. Within a year of life, these $Adh5^{-/-}Csb^{-/-}$ mice also develop neurodegeneration and chronic kidney failure. $Adh5^{-/-}Csb^{-/-}$ mice therefore reveal a phenotype that resembles CS, suggesting that chronic endogenous DNA damage caused by formaldehyde requires TC-NER in neurons and nephrons. Endogenous formaldehyde may therefore be a significant source of endogenous DNA damage that is repaired by CSB through TC-NER. Inability to respond to such damage may contribute to the clinical consequences of CS.

Oo9
Why DNA repair defects are not sufficient to explain the complex phenotype of Cockayne syndrome

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Cockayne syndrome (CS) subtypes encompass an expanding spectrum of clinical features, and yield a very large but continuous spectrum of clinical severities. The molecular causes of these defects are still largely obscure. On one end is UV sensitivity syndrome (UVSS), which displays UV hypersensitivity but no neurodegeneration and no progeroid defects characteristic of CS, despite resulting from mutations in *CSA* or *CSB*, as does CS. UVSS *de facto* uncouples the DNA repair defect from the other dramatic impairments of CS. In other cases, CS was not associated with clinical UV-hypersensitivity, further challenging the link between unrepaired DNA damage and CS. There is increasing evidence that multifunctional proteins, including DNA repair proteins, are implicated in diseases with complex phenotypes. CSA and CSB are involved in the repair of UV-induced DNA damage and also in other types of DNA repair. Moreover, CSA and CSB function as chromatin remodelers and transcription factors, and have been detected in mitochondria. We hypothesize that these proteins are causative of CS defects, in large part because of other functions than DNA repair. In this context, we recently demonstrated that CS patient-derived fibroblasts display reduced mitochondrial respiration due to depletion of the catalytic subunit of the mitochondrial DNA polymerase, in turn resulting from accumulation of the serine protease HTRA3.[1] HTRA3 overexpression in CS cell depends on nitrosative and oxidative stress, but the underlying mechanism is still unknown. Importantly, these defects were fully rescued in patient fibroblasts using a porphyrine derivative that scavenges reactive oxidative/nitrosative species. These alterations were unrelated to UV-hypersensitivity, since they were not detected in UVSS cells and were assessed in the absence of UV damage in CS cells. Mitochondrial dysfunction has been detected in CS cells and it has been largely ascribed to impaired processing of DNA damage and oxidative stress. In our paradigm, mitochondrial dysfunction in CS rather results from improper degradation of functional mitochondrial proteins. A large spectrum of abnormalities, progressive deterioration, nearly-identical mutations that may not produce identical diseases, like in CS, are indeed hallmarks of mitochondrial diseases.We will discuss evidences that CSA or CSB deficiency results in dramatic modifications that may affect mitochondrial and related functions. These alterations are possibly responsible for the plethora of clinical symptoms in CS and are not ascribable to DNA damage-related transcription defects. Understanding molecular defects in CS is essential to build up a rationale for ameliorating and/or rescue strategies.

1. Chatre L, Biard DS, Sarasin A, Ricchetti M. *Proc Natl Acad Sci U S A* 2015; 112: E2910–19.

Posters

Poo1
Maintenance of pericentric heterochromatin following UVC damage

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We live under the constant threat of agents such as chemicals and radiation, including UV radiation in sunlight, which

generate lesions in our DNA. DNA damage challenges not only genome stability but also the integrity of its organization with histone proteins into chromatin, which governs gene expression and cell identity. How chromatin organization is altered after DNA damage while preserving its functions is thus a central issue. In this report we tackle this question by focusing on heterochromatin domains, which pose a strong barrier to DNA damage repair and display high mutation rates in human cancers.[1] We test the possibility that dedicated mechanisms may have evolved for maintaining heterochromatin structure and function in response to DNA damage. For this, we have established a unique cellular model in mouse fibroblasts, allowing simultaneous tracking of UVC damage repair and associated histone dynamics in pericentric heterochromatin domains. Thus, we have observed that UVC damage repair takes place efficiently within pericentric heterochromatin, concomitantly with *de novo* deposition of H3.3 histones. Our findings also provide insights into the underlying molecular mechanisms, with the characterization of H3.3-specific chaperone accumulation in damaged heterochromatin. Furthermore, our observations indicate that chromatin modifiers and histone chaperones cooperate in heterochromatin maintenance following UVC damage. We are currently exploring the consequences of DNA damage-induced chromatin alterations on pericentric heterochromatin function. Altogether, the findings ofthis study should shed new light on the fundamental mechanisms involved in the maintenance of higher-order chromatin structures following DNA damage1. Fortuny A, Polo SE: The response to DNA damage in heterochromatin domains. *Chromosoma* 2018; **27:** 291–300.

P002
Repair protein persistence at DNA lesions characterizes xeroderma pigmentosum–Cockayne syndrome complex

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The structure-specific ERCC1-XPF endonuclease plays a key role in DNA damage excision by nucleotide excision repair (NER) and interstrand crosslink repair. Mutations in this complex can either cause xeroderma pigmentosum (XP) or XP combined with Cockayne syndrome (XP-CS complex) or Fanconi anaemia. However, most patients carry compound heterozygous mutations, which confounds the dissection of the phenotypic consequences for each of the identified XPF alleles. Here, we analysed the functional impact of individual pathogenic XPF alleles on NER. Wefound that XP-causing mutations diminish XPF recruitment to DNA damage and only mildly affect global genome NER. In contrast, an XP-CS complex-specific mutation causes persistent recruitment of XPF and the upstream core NER machinery to DNA damage and severely impairs both global genome and transcription-coupled NER. Remarkably, persistence of NER factors at DNA damage appears to be a common feature of XP-CS complex cells, suggesting that this could be a determining factor contributing to the development of additional developmental and/or neurodegenerative features in patients with XP.

P003
A Rare Disease Centre: a model for improved service delivery

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A rare disease is defined as a condition rgR h affects less than 1 in 2000 of the general population. Although rare diseases are individually rare, collectively they are common, with one in 17 people in the UK population having a rare disease at some point in their lives, 75% of whom are children. The publication of the UK Strategy for Rare Diseases[1] represented a significant milestone for patients with these conditions. The overarching aim of the strategy was to empower those affected by rare diseases, to facilitate early diagnosis and intervention, and to improve coordinated care. Guy's & St Thomas' NHS Foundation Trust has been at the forefront of developing a coordinated clinical approach to rare diseases, culminating in the establishment of a bespoke Rare Diseases Centre (RDC). This has enabled clinicians and scientists working in multidisciplinary teams to deliver a personalized, comprehensive package of care to meet the complex clinical needs of patients and their families. We will share our experience of developing integrated pathways to improve patient care in the RDC, including the many challenges in developing this unique centre. The importance of patient and family input into service development will be highlighted, alongside the opportunities provided for translational research.

1. Department of Health. UK Strategy for Rare Diseases. Available at: https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/260562/UK_Strategy_for_Rare_Diseases.pdf

P004
Transcription factor IIE orchestrates the recruitment of the transcription factor IIH kinase module at promoter before their release during transcription

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Trichothiodystrophy (TTD) is an autosomal recessive developmental disorder mainly related to mutations in transcription factor II H (TFIIH), a general transcription factor also involved in DNA repair. Remarkably, mutations within the β subunit of transcription factor II E (TFIIE), another general transcription factor, have been recently associated with TTD, which prompted us to accurately dissect the partnerships occurring between TFIIE and TFIIH during transcription. Our work revealed an unexpected dynamic process during which TFIIE acts as key factor to recruit and position the Cdk-activating kinase (CAK) module of TFIIH within the preinitiation complex. Furthermore, we observed that the Pol II phosphorylation is accompanied by the release of the CAK module and

TFIIEα from the promoter, a process that takes place before DNA opening. While RNA synthesis is initiated, the Core-TFIIH and TFIIEβ are also removed and elongation factors including DSIF are recruited. Strikingly, TTD-related mutations in either *XPD* or TFIIEβ coding gene (*GTF2E2*) disrupt these early transcriptional processes similarly, which could explain why alterations of TFIIE or TFIIH lead to the same clinical syndrome.

P005
XPD mutations differentially affect the association of the cyclin-dependent kinase-activating kinase and core transcription factor iIH subcomplexes and their interaction with chromatin

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Transcription/DNA repair factor IIH (TFIIH) is made of two distinct subcomplexes, named core TFIIH and cyclin-dependent kinase (CDK)-activating kinase (CAK), bridged together by the XPD subunit. The CDK7 kinase subunit of the CAK subcomplex targets different substrates: as free CAK it phosphorylates specific CDKs and promotes cell cycle progression, while as a subunit of the TFIIH complex, CDK7 phosphorylates and activates RNA polymerase II and specific transcription factors. In this context, the bridging factor XPD plays a key role in modulating the association/dissociation between CAK and core TFIIH, thus linking transcription to cell cycle control. Mutations in the *XPD* gene are responsible for distinct clinical entities, including the cancer-prone condition xeroderma pigmentosum (XP) and the multisystemic cancer-free condition trichothiodystrophy (TTD). To understand whether *XPD* mutations affect the composition of TFIIH complex and, in turn, the CAK-mediated signalling pathways, we investigated the association/dissociation dynamic of the two TFIIH subcomplexes throughout the cell cycle. Native chromatin immunoprecipitation and immunofluorescence studies revealed altered cellular distribution and chromatin association of CAK and core TFIIH in XP or TTD cells, suggesting the alteration of CAK-dependent signalling pathways in the fibroblasts of patients with XP-D.

P006
Exploring the function of TTDN1 in genome maintenance and gene expression

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The rare recessive trichothiodystrophy (TTD) syndrome is characterized by the presence of brittle hair, caused by low amounts of sulphur-rich proteins in hair-shaft keratinocytes. TTD is caused by mutations in *XPD*, *XPB*, *GTF2H5/TTDA*, *GTF2E2* *RNF113A* or *MPLKIP/TTDN1* genes. The function of TTDN1 is still unknown and the protein has no obvious link with either DNA repair or gene expression. We performed stable isotope labelling by amino acids in cell culture (SILAC)-based quantitative mass spectrometry to identify TTDN1-interacting proteins, using TTDN1–green fluorescent protein (GFP) knock-in cells to immunoprecipitate interacting complexes under mild conditions. The obtained TTDN1 interactome suggests that TTDN1 may function in RNA processing as it shows strong interaction with: (i) XAB, (ii) AQR, PPIE and ISY1, and (iii) the CWF and DBR1 complex. Initial genotoxicity screening experiments revealed that TTDN1-depleted cells are surprisingly hype-sensitive to the DNA interstrand-crosslinking (ICL) agent mitomycin C. Live cell imaging showed a striking accumulation of TTDN1-GFP to locally generated (subnuclear) DNA damage, induced by multiphoton laser, further suggesting a function of TTDN1 in DNA repair.

P007
ONKOTHER-H: Development platform for innovative therapies using the example of the most frequent human cancer, skin cancer

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ONKOTHER-H is a joint project funded by the European Social Fund as part of the Excellence Research Program of Mecklenburg-Vorpommern in Germany. This consortium aims at establishing a translational development platform for novel cancer therapies. The most frequent human cancer – skin cancer – serves as a well-established model for innovative cancer therapies. New pharmacologically relevant small molecules (isatines and oxindole derivatives) and innovative treatment with cold atmospheric pressure plasma as well as the combination of both will be evaluated *in vitro* and *in vivo* using cutaneous melanoma and skin squamous cell carcinoma as examples. As part of this joint project, our subproject focuses on the effect of novel therapies on genotoxicity and DNA repair. After determination of inhibitory doses at which 50% of cells die, we quantify the amount of reactive oxygen species using oxidation of H_2DCFDA as an indicator. With the help of specific antibodies, oxidative DNA lesions [e.g. 8-oxo-guanine (8-oxo-G], dimerization [pyrimidine-pyrimidone photoproducts (6-4PP), cyclobutane pyrimidine dimers (CPD)] and DNA crosslinks (anti-cisplatin-modified DNA CP9/19) are quantified. DNA double-strand breaks are quantified using gamma-H2AX detection. Furthermore, the DNA repair capacity of the cells is assessed using a host cell reactivation assay and compared with the cellular toxicity of the respective treatment. A plasmid shuttle vector assay is used to quantify the mutagenicity and toxicity of the treatment in comparison to UV irradiation. It is expected that results from this subproject together with results from other subprojects will bring about novel cancer therapies and establish a development platform for further innovative anticancer therapies.

P008
Modelling nucleotide excision repair-related diseases using human induced pluripotent stem cells.

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DNA damage occurs continuously in our cells. Thanks to DNA repair pathways, most of the newly occurring DNA damage is repaired. The nucleotide excision repair (NER) system is one of the most important DNA repair pathways. NER is involved in the repair of a variety of DNA-distorting structures such as UV-induced pyrimidine dimers, intra-strand crosslinks and bulky DNA adducts. A defect in one of its two subpathways, global genome nucleotide excision repair (GG-NER) or transcription-coupled nucleotide excision repair (TC-NER) may lead to severe phenotypes including early-onset cancer, sensitivity to UV and/or neurodegeneration. Xeroderma pigmentosum (XP) and Cockayne syndrome (CS) are examples of such clinical syndromes caused by the deficiencies of the TC-NER and GG-NER pathways, respectively. It is well known that terminally differentiated cells such asneurons exhibit an attenuated NER function, which makes those nondividing cells very vulnerable in patients with XP or CS. However, the molecular mechanisms behind this remains poorly understood. In order to establish a clear genotype–phenotype correlation for the neurodegeneration observed in XP and CS diseases, we generated human induced pluripotent stem cells (hiPSCs) from patients carrying different XP and CS mutations. More than 15 hiPSC cell lines derived from patients and matched healthy controls, as well as clustered regularly interspaced short palindromic repeat (CRISPR)-cas9-KO-edited hiPSCs were generated and characterized. The different hiPSCs were then efficiently differentiated into neural stem cells (NSCs) and neurons. Here, we present the preliminary results of differentiation capacity, neuronal morphology and -omics data.

P009
The sequential and cooperative mechanism of transcription-coupled repair complex assembly provides insight into the molecular origin of Cockayne syndrome

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Transcription-coupled nucleotide excision repair (TCR) ensures the removal of transcription-blocking DNA lesions from actively transcribed strands. These lesions are recognized by the stalling of RNA polymerase II (RNAPII), followed by recruitment of the TCR factors (Cockayne Syndrome A and B (CSA, CSB) and UV-stimulated scaffold protein A (UVSSA)], after which the core DNA repair complex, including transcription factor IIH (TFIIH), is assembled. Interestingly, mutations in the *CSB* and *CSA* genes cause Cockayne syndrome (CS), while mutations in *UVSSA* cause the very mild UV sensitivity syndrome (UVSS). Understanding the assembly mechanism and function of TCR proteins will not only define the molecular mechanism of TCR, but will also provide new insight into the molecular origin of CS. To study the TCR assembly mechanism, we generated a collection of human TCR knockout (KO) cells and established new immunoprecipitation-based methods. Our findings show that CSB mediates the targeting of CSA and UVSSA to RNA polymerase II (RNAPII). While CSA is dispensable for CSB recruitment, it is essential for the targeting of UVSSA. Finally, we found that the sequential assembly of CSB, CSA and UVSSA is highly cooperative and that each factor is required for the efficient recruitment of the TFIIH complex to initiate repair. To identify the region in CSB that is required for the interaction with CSA, we used a chromatin-tethering approach in which CSB mutants fused to the bacterial LacR were targeted to LacO repeats to visualize the enrichment of GFP-tagged CSA. We founnd that the C-terminal region of CSB, specifically amino acids 1385–1399, is essential for the interaction with CSA. We are currently functionally characterizing CSB-KO cells stably expressing GFP-tagged CSBΔ1385-1399 using our IP-based methods. In order to further examine the role of UVSSA in TCR complex assembly, we complemented UVSSA-KO cells with green fluorescent protein (GFP)-tagged UVSSA separation-of-function mutants that are selectively impaired in their interaction with either CSA (UVSSAΔ100-200) or the TFIIH complex (UVSSAΔ400-500). Indeed, we found that UVSSAΔ100-200 fails to associate with stalled RNAPII, whereas UVSSAΔ400-500 is targeted to stalled RNAPII, similar to wild-type UVSSA. Interestingly, both mutants fail to recruit the TFIIH complex, demonstrating that UVSSA is the key factor that recruits TFIIH upon its targeting to RNAPII by CSB and CSA to initiate repair. Together these findings reveal the sequential and highly cooperative mechanism of TCR complex assembly, and suggest that the primary cause of CS may not be a defect in the removal of transcription-blocking DNA lesions.

P010
The role of the xeroderma pigmentosum (XP) clinical nurse specialist in the U.K.

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In 2011, two xeroderma pigmentosum (XP) clinical nurse specialists (CNSs), one for adults and one for children, were appointed. These nurses work collaboratively within the multidisciplinary XP team. A CNS is a nurse who is educated to degree level or above, and holds specialist knowledge, skills, competencies and experience. This XP job is unique to the UK. These nurses are responsible for the setting up of the nursing outreach, visiting patients in their own homes.The nurse input into the following will be demonstrated: (i) patient communication and support, (ii) home visiting, (iii) school and workplace visiting, (iv) patient, public and peer education (v) research, (vi) clinics and (v) service development. Measurable outcomes include the following: 100% of patients have been offered a home visit, and 67% adults and

94% of children have been visited; 100% of schoolchildren have been offered a school visit, and 96% have been visited; 26% of adults in 2011 previously had ultraviolet (UVR) protective window film on their home windows, and this has increased to 60%; 82% of children in 2011 previously had UVR protective window film on their home windows and this has increased to 97%; and 67% of schools with a pupil with XP ad UVR protective window film in 2011, and this has increased to 94%. '*We feel the service has been enhanced since the appointment of the two Specialist Clinical Nurses. The feedback from families regarding the Nurses has been exceptional.*' (*XP Support Group* 2018). At present, there is no known cure for XP so to continually engage a patient on an individual personalized basis, along with those around them, in maximizing their understanding and ability to help themselves is important. An XP CNS is in an ideal position to be able to facilitate this. In conclusion, a dedicated service set-up caring for patients with XP benefits from having the input of CNSs.

P011
Neurological phenotyping and disease progression in xeroderma pigmentosum: preliminary results in complementation groups D and G

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Xeroderma pigmentosum (XP) is characterized by neurological features in 20–50% of patients. The phenotype shows substantial variability between the complementation groups and between patients in a single group. XP can present with a combination of cognitive impairment, cerebellar ataxia (CA), pyramidal signs, neuropathy, sensorineural hearing loss (SNHL) and movement disorders. Such heterogeneity complicates the use of rating scales to quantify disease progression. We present the neurological phenotype of two complementation groups (XPD and XPG) and the potential role of several clinical tools in the assessment of disease progression.Patients from a specialized service were followed for a minimum of 3 years. Clinical variables were collected in those visits and from previous reports. The neurological examination was recorded with the Scale for Assessment and Rating of Ataxia (SARA) and the Inventory of Non-Ataxia Signs (INAS). The first visit with a complete SARA score was selected as baseline. For the follow-up visit, we considered the 3-year visit if a full SARA score was available. In cases where SARA was incomplete, the nearest visit (± 1 year) was selected as the follow-up visit. SPSS software (v.24.0) was used for statistical analysis. In both groups, a high proportion of subjects presented with neurological features (XPD: 12/14; XPG: 7/8), with a median age of onset below 20 years and median disease duration above 10 years. Neurodevelopmental delay and SNHL were the earliest symptoms in XPD, whereas cognitive impairment was the initial symptom in XPG. Both groups showed CA, spasticity, distal limb weakness, oculomotor signs, urinary dysfunction and cognitive impairment. In XPD, there was a higher proportion of hyporeflexia (63.6% vs 12.5%, p=0.059) and of hypopallesthesia (40% vs 0%, p=0.231) compared with XPG. Conversely, hyper-reflexia (50% vs. 18.2%, P = 0.319) and chorea/dystonia (37.5% vs. 14.3%, P = 0.309) were more frequent in XPG. Baseline SARA and INAS scores were similar in the two groups. In XPD, the median SARA change was 2 points (range −1 to 10, P = 0.042), whereas no significant change was observed in XPG. Regarding INAS, XPD showed some tendency to progression (median change: 1.5 points, range −1 to 4; P = 0.104), whereas no definite change was observed in XPG. Neurological features are one of the hallmarks of XP. Despite some overlap, different patterns can be appreciated in patients with XPD and those with XPG. Our study suggests that SARA might be suitable to assess progression in some XP groups. We plan to perform a similar characterization for all the complementation groups.

P012
Xeroderma pigmentosum and vitamin D deficiency; how to manage it?

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Vitamin D is a fat-soluble compound essential for human life. The classic roles of vitamin D in maintaining bones and teeth health and preventing rickets in children and osteomalacia in adults are based upon its function in enabling calcium absorption from the small intestine. There are also many nonclassic roles for vitamin D in many tissues other than bone and the small intestine, including the pancreas, kidney, skin and selected cells of the immune system. Most (90%) of human vitamin D is produced in the skin through exposure to sunlight, mainly ultraviolet B radiation (UVB). Some foods are naturally high in vitamin D, such as oily fish and marine mammals. Patients with xeroderma pigmentosum (XP) are deprived of sun exposure because of the requirements of an indoor lifestyle, complete clothing coverage and sunscreen use imposed by the disease. In this paper, we report the results of the level of vitamin D in the blood of 23 patients with XP; 11 males and 12 females, aged between 2 and 48 years. The results showed that 22 patients had a low rate of vitamin D; 14 of them had severe deficiency, with blood vitamin D level under 10 ng/mL, while the other eight had a deficit between 10 and 20 ng/mL. Only one patient had a normal Vitamin D level. Only three cases had a low blood calcium level, but they had no clinical signs. As there is no codified scheme, we recommended a diet rich in vitamin D and gave supplementation with vitamin D to our cases, as follows: 100 000 IU/month for 3 months, than the same dose every 3 months in the patients with severe deficiency, while the patients with deficit received 100 000 IU over 3 months. An increase in vitamin A

levels was obtained in our cases after 2 years of follow-up, but the small size of our series does not allow us to extract conclusions. Further studies on larger series with long follow-up are needed.

P013
GTF2E2 gene defect in a patient with trichothiodystrophy

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Trichothiodystrophy (TTD) is characterized by the common feature of sulfur-deficient brittle hair associated with variable neuroectodermal symptoms. This autosomal recessive disorder is genetically heterogeneous. Two forms of TTD are distinguished: a photosensitive form associated with DNA repair defects and a nonphotosensitive form with normal DNA repair.We describe a new patient with common features of nonphotosensitive TTD due to a missense variant, present in the homozygous state, in the GFT2E2 gene (coding for General transcription factor IIE). Similar to the only three patients described in the literature, the patient, aged 2 years, presented developmental delay with short stature and microcephaly. Her parents are first cousins and originate from Morocco, similar to the first patients described with this mutation. Functional studies in patients presenting GTF2E2 mutations have shown normal results for nucleotide excision repair (NER) testing and reduced expression of both subunits of the transcription factor IIE (TFIIE) complex (TFIIEα and TFIIEβ). This finding is consistent with the decreased phosphorylation level of TFIIEα observed in patients with the variants of ERCC2 associated with the TTD phenotype (XPD-TTD). This novel case of TTD linked to GTF2E2 gene defect in the same geographical area raises the possibility of an eventual founder mutation in Morocco and emphasizes careful hair examination as a useful guide for genetic analysis in patients with neuroectodermal disorders and prominent hair anomalies. The use of a multigene panel (next-generation sequencing) for genetically heterogenous disorders such as TTD reveals a potent tool that is able to speed up the diagnosis.

P014
Particular forms of xeroderma pigmentosum and Cockayne syndrome in the Tunisian population

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Xeroderma pigmentosum (XP) and Cockayne syndrome (CS) are rare autosomal recessive disorders that affect DNA repair process. XP is characterized by UV-induced skin dyspigmentation, sunburn, cancers and in some patients, neurological degeneration. The incidence of XP is relatively low in Europe (1 in 250 000), but is more frequent in Tunisia (1 in 10 000). CS is characterized by progressive neurological disorders, a progeroid appearance, microcephaly, severe psychomotor delay and intracranial calcifications. Clinical and molecular analysis has shown that the correlation between phenotype and genotype is difficult, which makes genetic diagnosis using conventional sequencing techniques costly and time-consuming. Here we describe seven patients from five families with CS including two siblings (without apparent photosensitivity) and two patients with a particular form of mild XP phenotype. Clinical and genealogical data were collected using a standardized survey for each suggestive phenotype. DNA and RNA were extracted from blood samples. We performed targeted gene sequencing of DNA repair genes and direct sequencing of cDNA to confirm the molecular impact of the detected variation. For XP, we identified for the first time the XPG complementation group in Tunisia and North Africa. The patients were carriers of an homozygous T>C transition at nucleotide position c.2333 causing a leucine to proline amino acid change at position 778 (p.Leu778Pro) of the ERCC5gene, resulting in an XPG phenotype. The same variant has been previously reported in the heterozygous state in a patient cell line in Europe, for which no clinical data were available and which was suggested to confer a XP/CS phenotype. For CS, we identified a causative mutation (p.Tyr200Lysfs*12) in the ERCC8 gene in patients carrying typical characteristics of CS. This mutation seems to be specific to North African people. Interestingly, targeted gene sequencing for two CS siblings with apparent lack of UV hypersensitivity showed a novel splice site mutation in the ERCC8 gene. We are performing unscheduled DNA synthesis (UDS) and recovery of RNA synthesis (RRS) tests to confirm the functional effect of this identified mutation. This study extends the mutation spectrum of rare DNA repair diseases such as XP and CS. Defects in the ERCC5gene remain a paradigm in DNA repair

diseases. Indeed, mutations in this gene can result in phenotypes that range from the mild XP form to the most severe combined form of XP/CS. Reinforcing previous observations, these data reveal that photosensitivity may not be a fundamental criterion to characterize CS. Both XP and CS are models for premature ageing studies.

P015
Transcription in *Xenopus* egg extract; towards recapitulating transcription-coupled DNA repair

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Many types of DNA damage impair the elongation of RNA polymerase II (RNAPII), creating a need for transcription-coupled DNA repair (TCR) pathways. In transcription-coupled nucleotide excision repair (TC-NER), RNAPII that is stalled at a lesion such as a UV-induced thymine dimer is detected by the proteins CSA and CSB. This results in the recruitment of the nucleotide excison repair (NER) machinery and subsequent excision and replacement of the damaged strand. However, the exact sequence of biochemical events triggering TC-NER remains elusive, largely due to the absence of a cell-free system that supports TCR. Moreover, even less is known about TCR mechanisms of more complex lesions including highly cytotoxic DNA interstrand crosslinks (ICLs) and DNA–protein crosslinks (DPCs). *Xenopus* egg extracts are a powerful, highly tractable, cell-free system that faithfully recapitulates a wide range of cellular processes including DNA replication and repair. These extracts have been instrumental in elucidating the intricacies of replication-coupled ICL and DPC repair. However, egg extracts are widely considered to be transcriptionally quiescent, which has prevented exploring TCR pathways in this system so far. Here, we report the first robust *Xenopus* egg extract-based *in vitro* transcription system. Using a plasmid containing a viral promoter flanked by multiple activation sequences, we show that highly concentrated nucleoplasmic egg extract supported RNAPII transcription in an activator-dependent manner, whereas total egg lysate was inactive. In an effort to recapitulate TCR, a panel of site-specific DNA lesions was introduced downstream of the promoter region. While RNAPII stalled at the damage, TCR was not observed. In contrast, transcription-independent global-genome nucleotide excision repair (GG-NER) occurred, suggesting that recognition of stalled RNAPII is defective. Subsequent analysis revealed that CSA and CSB are present at very low levels in egg extracts, being efficiently expressed or stabilized only after zygotic transcription is activated during the mid-blastula transition. This observation is consistent with our finding that CSA and CSB are not recruited to damage-stalled RNAPII and the absence of TCR in egg extract. To overcome this limitation, we recombinantly produced the CSA-containing E3 ubiquitin ligase complex as well as CSB and we are now supplementing the cell-free system with these proteins. We will present our progress towards the activation of TCR in *Xenopus* egg extract, and discuss advantages and additional future applications for this novel *in vitro* transcription system.

P016
Two siblings with near-complete nucleotide excision repair inactivation due to inherited ERCC1 deficiency displaying mild clinical features of xeroderma pigmentosum and Cockayne syndrome with severe liver impairment

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Nucleotide excision repair (NER) is the major pathway to remove helix-distorting DNA lesions from the genome through a dual incision mechanism involving the ERCC1/XPF and XPG endonucleases. In addition to NER, the ERCC1-XPF endonuclease also has important roles in other DNA repair pathways, such as interstrand crosslink (ICL) and DNA double-strand break (DSB) repair. Inherited defects in NER can result in xeroderma pigmentosum (XP), a syndrome that is characterized by extreme photosensitivity and highly increased skin cancer risk, or in Cockayne syndrome (CS), which is a segmental progeria characterized by severe neurodegeneration. Patients with inherited ERCC1 deficiency are rare, and only two such patients have been described to date (165TOR and CS20LO), who both displayed skeletal abnormalities and developmental defects causing early childhood death (at 1 and 2.5 years, respectively). The clinical phenotype of those patients with ERCC1 deficiency, in particular 165TOR, was distinct and more severe than NER deficiency alone, which suggests that other functions of ERCC1 outside NER contribute to this severe phenotype. We describe two siblings (10 and 12 years old) with inherited ERCC1 deficiency showing mild cutaneous photosensitivity and corneal burning without apparent skin cancer and very mild features of CS. Surprisingly, both patients displayed progressive cholestatic liver impairment and received successful liver transplants. Primary fibroblast obtained from both these patients with ERCC1 deficiency (XP46PV and XP50PV) revealed very low expression levels of ERCC1 protein, which contained a missense variant within the XPA-binding domain. Both unscheduled DNA synthesis (UDS) and recovery of RNA synthesis (RRS) analyses revealed near-complete inactivation of NER, almost to the same level as that detected in NER-deficient XPA fibroblasts. We are currently testing, in patient-derived fibroblasts and reconstituted ERCC1 knockout cells, to what extent the identified ERCC1 missense variant still supports ICL and DSB repair. Moreover, we are mapping the interactome of this ERCC1 variant in response to a variety of DNA-damaging agents by mass spectrometry. In conclusion, we report two new patients with ERCC1 deficiency with near-complete inactivation of NER, showing rather mild features of XP and CS, but displaying severe liver impairment. We propose that the ERCC1 mutations in these patients represent a separate-of-function variant that selectively

impairs NER leading to mild XP and CS, and that liver impairment might be an ERCC1-specific feature of which the origin is currently unknown.

P018
Distinctive transcriptional signatures between trichothiodystrophy and xeroderma pigmentosum

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transcription factor IIH (TFIIH) is a multi-protein complex involved in transcription and in the nucleotide excision repair (NER) pathway. Mutations in genes encoding the XPD and XPB subunits of TFIIH are responsible for rare inherited disorders, including xeroderma pigmentosum (XP) and trichothiodystrophy (TTD). Besides sharing skin photosensitivity, patients with XP and those with TTD exhibit distinct clinical features. XP is characterized by high incidence of cutaneous precancerous lesions and cancers, in association with progressive neurological degeneration. TTD shows hair abnormalities, ichthyosis, physical and mental retardation, proneness to infections and signs of premature ageing, but not cancer predisposition. Resulting from distinct mutations in the same causative genes, XP and TTD represent good models to dissect the signalling pathways implicated in either carcinogenesis, neurodegeneration or ageing. By taking advantage of the next-generation sequencing technology, we analysed the whole transcriptome of primary dermal fibroblasts from patients with TTD or XP, with mutations in *XPD*. Our results revealed wide transcriptional impairments both in basal condition and in response to UV irradiation in XP and TTD cells. Strikingly, TTD fibroblasts presented a higher number of deregulated genes compared with XP fibroblasts, the majority of which were transcriptionally downregulated. Moreover, by extending the expression analysis to a larger number of patients with TTD s as well as to a TTD mouse model, we identified a restricted number of genes that are systematically deregulated in TTD and define a specific transcriptional signature for the TTD phenotype. Finally, by further investigating the consequence of such transcriptional alterations, we observed reduced levels of specific proteins in TTD fibroblasts, which can be considered as novel diagnostic biomarkers for this disorder.

P019
Molecular and functional analysis of a splice mutation in ERCC6

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Cockayne syndrome (CS) is an autosomal recessive disorder caused by pathogenic mutations in *ERCC6/CSB* or *ERCC8/CSA*. Common signs of this multi-organ-affecting disease include severe growth failure, cutaneous photosensitivity, progressive hearing loss, cataracts, progressive neurodegeneration and premature ageing. We performed whole exome sequencing on DNA derived from a novel patient and identified a homozygous *ERCC6* splice variant, c.1992+3A>G. We functionally characterized patient-derived primary fibroblasts for their ability to repair UV- induced DNA lesions. We demonstrate that primary fibroblasts are hypersensitive to UV irradiation and are defective in the transcription coupled nucleotide excision repair subpathway.

P020
A single ubiquitylation site on RNA polymerase II couples DNA damage response to transcription

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In response to transcription-blocking DNA damage, cells orchestrate a multi-faceted reaction that includes transcription-coupled nucleotide excision DNA repair (TC-NER), and also degradation of RNA polymerase II (RNAPII) and a dramatic genome-wide transcriptional response, involving temporary transcription shutdown. If and how these responses are connected has remained unclear. We show that DNA damage-induced ubiquitylation of RNAPII itself, at a single lysine (K1268 of RPB1), serves as the focal point for response coordination. When this target lysine is mutated to arginine (K1268R), DNA damage-induced RNAPII poly-ubiquitylation and degradation are completely prevented and cells become UV-sensitive. Intriguingly, K1268R cells display a delay in repair of transcription-blocking DNA lesions, but this seems to occur independently of TC-NER. Instead, the inability to degrade K1268R RNAPII at the sites of damage obscures access to general genome repair factors. Our data strongly support the idea that RNAPII ubiquitylation and degradation thus serve as the 'last resort' to remove lesion-stalled RNAPII complexes that fail to initiate/complete TC-NER. Additionally, we reveal that K1268 ubiquitylation is essential for a normal transcriptional response to UV irradiation: thousands of genes that should have been turned off remain expressed or even become hyperinduced in K1268R cells. Remarkably, all these misregulated genes are short (less than 25 kb long). Using mathematical modelling, we show that cells require RNAPII degradation upon UV irradiation in order to prevent redirection of RNAPII molecules from long genes (which have a high probability of containing transcription-blocking DNA lesions and are thus not permissive for completion of transcription) to short genes (low probability of DNA lesions, thus allowing completion of transcription). Besides revealing RNAPII itself as a key signal integrator in the DNA damage response, these data show that UV-induced global transcription shutdown is regulated by RNAPII degradation and point to the important role played by this phenomenon in cellular survival after DNA damage.

P021
Coordinated activity of Y family translesion polymerases and exonuclease 1 protects non-cycling cells from cytotoxic lesions

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In the human body, many cells are postmitotic or terminally differentiated. Depending upon their specific tissue origin, different sources of bulky lesions (e.g. UV light, chemotherapeutics, cigarette smoke, pollution) can compromise the stability of the genome of differentiated cells. We demonstrated that DNA damage checkpoint response in UV-irradiated non-cycling cells requires a coordinated activity of nucleotide excision repair (NER) and exonuclease 1 (EXO1). EXO1 generates long tracts of single-stranded DNA in response to bulky adducts at a subset of lesions, whose repair is not efficiently completed by canonical NER. The nature of the lesions requiring EXO1 activity is still unknown. We proposed that clusters of lesions affecting both DNA strand (closely opposing lesions, COLs), may be problematic for NER and may require EXO1 intervention. Accordingly, we observed that Y family trens lesion (TLS) polymerases are recruited at NER- and EXO1-positive sites. Polymerase κ and ι recruitment is dependent upon EXO1 activity, while Polymerase η and REV1 recruitment is not. Interestingly, if recruitment of TLS polymerases and their activity are inhibited, we observed hyperactivation of DNA damage checkpoint and generation of double-strand breaks, deriving from the uncontrolled processing of NER intermediates by EXO1. Balanced activity of TLS Pols and EXO1 downstream of NER is crucial to prevent unscheduled cytotoxic lesions formation after removal of DNA bulky adducts. Molecular details about NER, TLS Pols and EXO1 action after induction of bulky lesions from different sources will be explored in physiologically noncycling cells.

P022
Genetic profile of Brazilian patients with nucleotide excision repair deficiency

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Human syndromes resulting from deficiencies in nucleotide excision repair are known to display photosensitivity and/or neurological problems, such as xeroderma pigmentosum (XP), Cockayne syndrome (CS) and trichothiodystrophy (TTD). In Brazil, a few case reports havee been described, but genetic and molecular characterization data are scarce. Next-generation sequencing (NGS) provides the possibility to identify mutations directly. We initiated a project to diagnose the mutations involved in NER syndromes in Brazilian patients. To date, we are aware of 170 Brazilian patients with XP, which would give us a frequency of approximately 1 in 1 million inhabitants, similar to Western European populations. Since 2010, our group has received, 160 samples; 82 with a clinical diagnosis of XP, four with TTD and one with CS, along with 69 samples from relatives of these patients and four from patients with predisposition to skin cancer but no clinical diagnose. From this cohort, we have identified the germline mutations in 3382 patients with XP and 1/4 patients with TD. Along with us, the A.C. Camargo Cancer Center n São Paulo already identified the mutations from 32 other patients.[1] From both cohorts, there are currently 72 new Brazilian patients genetically characterized, of which the majority are XP-V or XP-C. From the DNA repair cohort, 2/32 have mutations at XPA, 5/32 at XPE-DDB2 12/32 at XPV-POLH and 1414/32 at XPC, while the patient with TTD patient has a mutation in XPD-ERCC2. Interestingly, 11 of 16 mutations are novel. It is important to highlight that most of the patients with XP-C patients carry a mutation previously found in the Comorian Islands, close to Mozambique in the Indian Ocean.[2] The molecular diagnosis of these patients offers not only data for the distribution of mutations of NER patients in Brazil, but also gives the patient and their families the opportunity for genetic counselling and for better health and social support.Keywords: NER disorders, Genetic clusters, Brazilian patients.

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1.Santiago *et al.*, 2019, submitted.

2. Cartault F, Nava C, Malbrunot AC *et al. DNA Repair (Amst)* 2011; 10: 577–85.

P023
Exploring retinal cone system function in a cohort of patients with xeroderma pigmentosum using electrophysiology

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Introduction Xeroderma pigmentosum (XP) is a rare genetic skin disorder whereby UV-damaged DNA is unable to repair itself correctly. There are approximately 100 documented cases in the U.K. ystemic and ocular abnormalities have been documented in certain subgroups of XP. Neurological abnormalities can occur, and in this study we explored retinal electrophysiology, which has not previously been studied. The aim of this study was to compare photopic electroretinogram (ERG) parameters from patients with XP with age-adjusted reference ranges. Photopic flash and flicker ERGs (corresponding to international standard stimuli) were recorded from consecutive patients with XP in the nationally commissioned U.K. XP clinic, using a portable device (RETeval, LKC Technologies Inc., Gaithersburg, MD, USA) and skin electrodes. The results were compared with an age-adjusted reference range supplied by the manufacturer. Two readings were attempted per eye and final readings from both eyes were averaged. Amplitudes and implicit times below the 2.5% reference limit and above the 97.5% reference limit were defined as abnormal. Patients with other concurrent retinal diseases such as diabetic retinopathy were excluded. In total, 42 consecutive patients with XP (mean ± SD age, 32.4 ± 18 years) were included (5 XPA; 13 XPC; 5 XPD; 5 XPE; 2 XPF; 4 XPG; 8 XPV subtype). Five patients (12%) had one or more photopic ERG parameters outside the normal range (one XPA, two XPC, one XPF and one XPG). The majority of patients with XP had photopic ERG parameters within the normal range. No patients with XPD, XPE or XPV had abnormal parameters. There have been no prior studies, and the possibility of abnormal retinal signalling in some XP subtypes invites further exploration.

P024
Thyroid anomalies in xeroderma pigmentosum

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Xeroderma pigmentosum (XP) is a rare autosomal recessive disease caused by a deficiency in the DNA repair system characterized by photosensitivity, pigmentary changes and a high incidence of UV-induced skin cancers. Extracutaneous signs have only rarely been studied. We describe a series of patients with XP presenting with a nodular disease of the thyroid. We reviewed all cervical ultrasonography scans performed as a work-up for squamous cell carcinomas of the head and neck in XP inpatients between January 2014 and May 2018. We divided them into a group with thyroid nodules (US+) and a group with no nodules (US-). In all cases, we noted the age, the sex, the complementation group and the geographical origin (coastal or inland). In total, 26 patients had undergone ultrasonography imaging during the past 4 years: 19 XPC (69.2%), 4 XPA (15.4%), 3 XPV (11.5%) and 1 XP with an unknown group (3.8%). The ultrasonography scans revealed thyroid nodules in 16 patients (61.5%) occurring mainly in the XPC group (13 patients, 81.3%), with one patient (6.2%) in each of the three other groups. The mean age of the patients was 16.92 years (range 6–53 years) with no difference between the US+ patients and the US- patients ($P = 0.9$). No significant difference was found between US+ and US- patients for sex ($P = 0.1$), geographical origin ($P = 0.054$) or complementation group (XPC versus non-XPC) ($P = 0.09$). Focusing on the XPC group, 72.2% had an abnormal ultrasonography scan. The mean age was 15 years (12.2 years in the US- group versus 16.1 years in the US+ group ($P = 0.015$). There was no significant difference in sex or geographical origin between the groups. Thyroid function tests performed in 16 cases (including all the cases of goitre) were normal. Thyroid anomalies in XP have only rarely been studied. An article published in 1999 reported a case of goitre and another of follicular carcinoma. In 2012, a French study published by the Sarasin team[1] found that out of 31 patients with XPC, 6 patients had thyroid anomalies. In our series, although thyroid abnormalities were more frequent in patients with XPC , no statistical difference was demonstrated, probably because of the low number of non XPC patients. In conclusion, our study reports a high frequency of thyroid abnormalities in XP. It also has the distinction of reporting this association in patients with XP other than group C. As discussed in the Sarasin study, thyroid function tests do not seem to be a good screening test.

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P025
Dermoscopic aspects of basal carcinomas in patients with xeroderma pigmentosum

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Xeroderma pigmentosum (XP) is a rare autosomal recessive disease characterized by the occurrence of multiple photo-induced skin cancers. Among these skin tumours, basal cell carcinoma (BCC) is the most common type. Dermoscopy is a noninvasive examination technique for early detection of these tumours. This work aimed to study the dermoscopic aspects of BCC in patients with XP and to correlate the dermoscopic signs with the histological subtypes of BCC. We conducted a prospective study from February 2014 to January 2017 at the Dermatology Department of Charles Nicolle Hospital, enrolling patients with XP presenting with histologically confirmed BCC for whom a dermoscopic examination was performed. We analysed 80 BCCs in 31 patients with XP:seven XPA (23%), 16 XPC (52%), six XPV (19%) and two XPE (6%). The average age was 23.48 years. BCCs were characterized clinically by the predominance of head and neck localization and presences of pigmented forms. Dermoscopic features included classical BCC patterns [blue-grey ovoid nests (95%), ulceration (95%), arborizing vessels (42%), multiple blue-grey globules (34%), maple leaf-like structures (37%) and spoke wheel-like structures (24%)]; structured patterns described as nonclassic BCC patterns (short fine telangiectasias (27%), multiple small erosions (4%)]; dermoscopic patterns commonly found in melanocytic lesions [blue-whitish veil (11%) and vascular polymorphisms (24%)]; and finally dermoscopic structures observed only under polarized light [structureless areas (40%), shiny white streaks (34%) and white circles (2%)]. The presence of blue–grey ovoid nests and maple-leaf-like structures were significantly associated with nodular BCC ($P = 0.01$, $P = 0.02$, respectively). Spoke-wheel-like structures and multiple small erosions were associated with superficial BCC ($P = 0.001$ for both signs). Yellow hyperkeratosis and dotted vessels were associated with basosquamous BCC ($P = 0.001$, $P = 0.02$, respectively). Our study illustrates the value of dermoscopy in the diagnosis of BCC in patients with XP and the correlation between dermoscopic features and histological subtypes, allowing the choice of a therapeutic modality before biopsy.

P026
Nicotinamide adenine dinucleotide supplementation prevents hearing loss in Cockayne syndrome mice

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Age-related hearing loss (ARHL) is the most common disorder affecting elderly populations, reaching up to 80% of individuals over the age of 85 years. Owing to its high prevalence, ARHL is considered a hallmark of ageing. Cockayne syndrome (CS) is a premature ageing disease with a very high rate of ARHL (up to ~84%) by the age of 12 years. CS is caused by mutations in the *CSA* and *CSB* genes, and hearing loss is a cardinal clinical symptom of the disease. Recent research revealed that hearing loss in CS is caused by defects in cochlear hair cells. These cells are highly metabolically active, making them particularly vulnerable to mitochondrial dysfunction. Interestingly, our laboratory demonstrated that cells from patients with CS manifest mitochondrial abnormalities, which can be reversed by supplementation with the essential metabolite NAD^+. Given that sensorial hair cells are high-energy-requiring cells, owing to their active metabolism, and that CS shows mitochondrial dysfunction, we hypothesized that NAD^+ repletion may ameliorate the hearing loss seen in CS through enhancement of mitochondrial homeostasis. To address this hypothesis, we examined hearing loss in CS mice by measuring electrical potential changes derived from the auditory brain stem, using auditory brainstem response (ABR). ABR is a novel technology to record brain wave activity and measure hearing thresholds in response to sound with different intensities (decibel; dB) and frequencies (Hertz; Hz). We found that CSB mice have increased hearing loss at 6 weeks of age (over 20 dB hearing loss at 32 kHz; $P < 0.05$). We then treated wild-type and CSB mice with the NAD^+ precursor, nicotinamide riboside (NR), in their drinking water. We observed that short-term NR treatment (approximately 10 days) significantly prevented hearing loss in CS mice and reduced the hearing threshold in response to sound from 65 dB to 40 dB intensity ($P < 0.01$) at high frequency. We then tested the effect of NR on the hearing defect in the CSA mouse and also detected improvement with short-term treatment (over 25 dB hearing loss at 32 kHz; $P < 0.001$). Cochlear histology analysis revealed that reduced presynaptic ribbon count in CSA and CSB cochlear inner hair cells was rescued with NR treatment at the base turn of cochlea, suggesting that NR improves hair cell innervation and functionality. Given that NR is a natural product with no known toxicities in mice, rats or people, it might be an effective intervention against hearing loss in mice and possibly in patients with CS and in older adults.

P027
Unravelling the pivotal role of DDA1 in transcription-coupled nucleotide excision repair

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DNA damage poses a serious threat to health, as it leads to mutations that cause cancer and interferes with replication and transcription, resulting in cell dysfunction and death, which contributes to ageing. Transcription-blocking DNA lesions are specifically targeted by transcription-coupled nucleotide excision repair (TC-NER), which is essential to protect against DNA damage-induced cellular toxicity, to safeguard genome stability and to preserve transcription programmes. Mutated TC-NER factors are associated with severe neurodegeneration and premature ageing. TC-NER is initiated by lesion-stalled RNA polymerase II, which subsequently triggers the assembly of several TC-NER-specific proteins, including the crucial TC-NER factor CSA. This protein is a component of CUL4-RBX1 E3 ubiquitin ligase complex required for the efficient progression of this vital process. Surprisingly, little is known about how CSA controls TC-NER. Using immunoprecipitations coupled to mass spectrometry, we uncovered a stable interaction between CSA and the DDA1 protein. DDA1 is a known subunit of the CRL E3 ubiquitin–ligase complex, and its knockdown/knockout affects nuclear targeting of CSA and inhibits TC-NER. Surprisingly, however, nuclear localization of DDB2 is not affected by depleting DDA1. DDB2 is another CRL-interaction partner that specifically functions in GG-NER. Moreover, live cell imaging experiments showed recruitment of DDA1 to DNA damage induced by UVC, dependent on transcription. Together our findings suggest that DDA1 is a newly identified TC-NER component. Our results provide new insights about protein interaction networks and how CSA is regulated to ensure proper repair of transcription-blocking lesions.

P028
Alternative excision repair pathway of UV-damaged DNA

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The nucleotide excision repair (NER) pathway is responsible for the removal of UV lesions, cyclobutane pyrimidine dimers (CPD) and pyrimidine (6-4) pyrimidone photoproducts (6-4PP). It has been believed that UV lesions are not recognized by single-strand break (SSB) repair (SSBR) involving the DNA repair protein XRCC1. Now we show genetic and biochemical evidence that SSBR removes at least 6-4PP. The loss of XRCC1 in XPA-deficient cells, but not wild-type cells, increases sensitivity to UV, delays removal of 6–4PPs and increases the size of alkaline comet tails, which are SSBR intermediates, following exposure to UV. In summary, XRCC1 contributes to repair of 6-4PPs independently of NER. We then explored the protein that triggers removal of 6-4PPs by SSBR. DNA topoisomerase I (TOP1) plays an essential role in DNA replication and transcription by resolving torsional stress. TOP1 cleaves one strand of a DNA double helix, allowing one end of the resulting SSB to rotate around the uncut strand prior to re-ligation of the SSB. TOP1 often fails to re-ligate when TOP1 cleaves near UV lesions, generating stable TOP1 cleavage complex (TOP1cc), i.e. TOP1 covalently associated with the 3— end of SSBs. Both Tyrosyl-DNA phosphodiesterase 1 (TDP1) and tyrosyl-DNA phosphodiesterase 2 (TDP2) accurately remove trapped TOP1 adducts from pathological TOP1ccs. We found that UV irradiation increases the number of pathological TOP1ccs in XPA-deficient cells but not wild-type cells. Inactivation of TDP1 and TDP2 increased UV sensitivity only in the absence of XPA. In summary, TOP1 activates SSBR, which can remove UV lesions when they are localized downstream of pathological TOP1ccs. A typical base excision repair starts by hydrolysing damaged bases forming abasic (AP) sites, followed by SSB formation 5′ to the AP sites. Repair synthesis from SSBs is carried out by the single-nucleotide (SN) and long-patch (LP) pathways, followed by ligation. Polβ contributes to LP repair undergoing strand-displacement DNA synthesis in collaboration with Fen1 endonuclease. We investigated whether TOP1-initiated SSBs stimulated LP repair synthesis and removed a CPD lesion *in vitro*. We chose CPD because it destabilizes the DNA helix to a lesser extent and thus more effectively represses the strand displacement synthesis compared with 6-4PP. We used a duplex DNA substrate (36 bp) with an SSB seven nucleotides upstream of CPD. We demonstrated that SSBR factors efficiently removes CPD. In conclusion, SSBR repairs 6-4PP and partially substitutes for lack of NER.

P029
Accelerated ageing in xeroderma pigmentosum: premature menopause, haematological malignancies and thyroid nodules

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Xeroderma pigmentosum (XP) is a rare autosomal recessive disease of DNA repair, characterized by severe ultraviolet (UV) sensitivity resulting in a 10,000-fold increased risk for skin cancer. XP is caused by mutations in any one of eight genes in the nucleotide excision repair (NER) pathway [*XPA*, *XPB* (*ERCC3*), *XPC*, *XPD* (*ERCC2*), *XPE* (*DDB2*), *XPF* (*ERCC4*), *XPG* (*ERCC5*)] or in the trans-lesion synthesis gene *pol eta* (XP-V).

In our National Insitutes of Health (NIH) natural history study, we have examined 149 people with XP since 1971 (age range 7 months to 79 years]. Symptoms include: freckling on sun-exposed skin before 2 years of age, severe burns after minimal sun exposure (about 50% of patients), skin cancers in children and UV damage to the eyes with loss of vision and development of ocular cancer. About 20% of patients developed progressive neurodegeneration, including sensorineural hearing loss, dysphagia and ataxia. Death from XP-related complications often occurred prematurely in the second or third decade of life. The median age of first skin cancer in our patients with XP was 9 years. This is a 50-year reduction for skin cancer compared with the general population and is evidence of a premature ageing phenotype. Recent improvements in medical care and UV protection have resulted in patients with XP living longer, active lives. We recognized three new nondermatological clinical findings. (i) Premature menopause (14 women); median age of menopause was about 30 years, which his 20 years younger than the general population; (ii) Haematological malignancies, which occurred in four patients (age range 18–36 years). Of these, two had myelodysplastic syndrome (MDS), one had giant B-cell lymphoma (BCL) and one had multiple phenotype acute leukaemia; development of MDS and BC usually occur between the ages of 50 and 70 years in the general population. (iii) Multinodular thyroid (17 patients; median age 25 years, age range 12–70 years); one patient had papillary thyroid cancer. Multinodular thyroid is seen most commonly in women age 35–50 years. These nondermatological findings have rarely been reported in patients with XP. In conjunction with the early age of skin cancer development, neurodegeneration and sensorineural hearing loss, these disorders are additional manifestations of a premature ageing phenotype in patients with XP.

P030
Histone deacetylase inhibition prevents loss of subcutaneous fat in a mouse model of Cockayne syndrome

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Cockayne syndrome (CS), a hereditary form of premature ageing predominantly caused by mutations in the *csb* gene, affects multiple organs including the skin, where it manifests with hypersensitivity towards ultraviolet (UV) radiation and loss of subcutaneous fat. There is no curative treatment for CS and its pathogenesis is only partially understood. Originally considered for its role in DNA repair, CSB protein most likely serves additional functions. Using CSB-deficient human fibroblasts, *Caenorhabditis elegans and* mice, we show that CSB promotes acetylation of α-tubulin and thereby regulates autophagy. At the organ level, chronic exposure of $csb^{m/m}$ mice to UVA radiation caused a severe skin phenotype with loss of subcutaneous fat, inflammation and fibrosis. These changes in skin tissue were associated with an accumulation of autophagic and lysosomal proteins and with reduced amounts of acetylated α-tubulin. At the cellular level, we found that CSB directly interacts with the histone deacetylase 6 (HDAC6) and the α-tubulin acetyltransferase MEC-17. Upon UVA irradiation, CSB is recruited to the centrosome where it colocalizes with dynein and HDAC6. Administration of the pan HDAC inhibitor suberoylanilide hydroxamine (SAHA) enhanced α-tubulin acetylation, improved autophagic function in CSB-deficient models from all three species and rescued the skin phenotype in $csb^{m/m}$ mice. HDAC inhibition may thus represent a therapeutic option for CS.

P031
The novel transcription factor IIH interactor helicase-like transcription factor stimulates nucleotide excision repair through regulating removal of excised oligonucleotides

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Nucleotide excision repair (NER) is a versatile DNA repair mechanism that protects the genome against a broad spectrum of helix-distorting DNA damage, including UV-induced DNA lesions. NER deficiency can lead to the development of cancer and/or accelerated ageing, underscoring the importance of the pathway in human physiology. The last decades many crucial insights have been obtained into how NER recognizes damaged DNA by XPC or by stalling of Pol II, and how transcription factor IIH (TFIIH) verifies the lesion and subsequently unwinds the DNA lesion in such a way that the DNA surrounding the lesion can be incised by the XPF/ERCC1 and XPG endonucleases. Even though it is an essential step for efficient restoration of the single-stranded gap by DNA synthesis, thus far it remains largely unknown how the incised, damage-containing oligonucleotide is removed from the chromatin. To identify factors involved in the eviction of damage containing oligonucleotides, we performed quantitative interaction proteomics using a cellular model in which a yello fluorescent protein (YFP)-tagged version of the largest TFIIH subunit, XPB, is expressed at endogenous levels. Interestingly, our results identified the helicase-like transcription factor (HLTF), a SWitch/sucrose non-fermentable (SWI/SNF) family chromatin remodeler that also possesses DNA helicase and ubiquitin ligase activities, as a TFIIH interactor. This UV-induced interaction is XPC- and XPG-dependent, suggesting that HLTF is recruited to TFIIH following incision. Using live cell imaging, we showed that HLTF knockdown results in prolonged chromatin binding following DNA damage to TFIIH, XPG and XPF. Despite the presence of the complete incision complex

upon HLTF depletion, we observed a reduced number of excised damage-containing oligonucleotides in the nucleoplasm, suggesting that HLTF might play a role in the efficient release of incised, damage-containing oligonucleotides. Efficient release of these oligonucleotides is expected to be essential for damage-induced DNA synthesis. In line with this, following HLTF depletion, recruitment of proliferating cell nuclear antigen (PCNA) to sites of DNA damage and gap-filling DNA synthesis (unscheduled DNA synthesis; UDS) are strongly reduced. Together, our results provide evidence that HLTF is required for proper progression through the NER reaction by stimulating the release of damage containing oligonucleotides from the chromatin, which most likely represent an important step for regulating the timely initiation of gap filling and DNA damage signalling.

P032
Matrix metalloproteinase-1 is more significantly upregulated by UVA-1 than UVC: mechanistic implications for the contribution of oxidative stress in the development of photoageing

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Photoageing studies have demonstrated the role of ultraviolet radiation (UVR)-induced upregulation of matrix metalloproteinases (MMPs). DNA damage, caused by both direct and indirect absorption of UVR photons [cyclobutane pyrimidine dimers (CPD) and oxidative stress, respectively] has been associated with skin MMP upregulation both *in vitro* and *in vivo*. However, the pathways through which photoageing occurs are unclear. Most diagnostic and mechanistic research on XP cells has used nonsolar UVC radiation; the effects of UVA, the major (> 95%) UVR component of sunlight, have been largely ignored. We wanted to identify a possible relationship between MMP (mRNA and protein) upregulation and CPD and/or reactive oxygen species (ROS) generation in XP compared with control fibroblasts, as a possible explanation for the early onset of photoageing observed in patients with XP. UVR spectral extremes and an antioxidant (vitamin E) were used to determine if XP fibroblasts would upregulate MMP more significantly after 254 nm UVC or 385 nm UVA-1 irradiation. We performed simultaneous irradiation of control and XP fibroblasts with 254 nm UVC and 385 nm UVA-1, followed by measurement of CPD, ROS and MMP at regular time points up to 24 hs postirradiation. For a given level of cell survival, 385 nm UVA-1 was a more effective inducer of MMP mRNA and protein than 254 nm UVC, by a mechanism independent of CPD. MMP upregulation was proportional to ROS generation by UVA-1, and this effect was attenuated by vitamin E, providing indirect evidence that ROS were the main cause of MMP upregulation. MMP-1 (collagenase) was more significantly upregulated in XP-C and XP-E fibroblasts compared with controls after UVA-1. The role of UVA in XP patients has been underinvestigated; our studies highlight that XP is a disease of impaired defences against direct and indirect UVR-induced damage, which may explain sunburn, photoageing and skin cancer. These studies demonstrate the importance of translational research in this rare genetic disease. Photoprotection from solar UVA, with antioxidants at the site of UVR absorption in the skin, is likely to be important for photoageing in the general population.

P033
DNA damage detection in nucleosomes register shifting

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Access to DNA packaged in nucleosomes is critical for gene regulation, DNA replication and repair. In humans, the ultraviolet DNA-binding protein (UV-DDB) complex detects ultraviolet light-induced pyrimidine dimers throughout the genome, yet it remains unknown how these lesions are recognized in chromatin, where nucleosomes restrict DNA access. Here we report cryoelectron microscopy structures for UV-DDB bound to nucleosomes bearing a 6-4 pyrimidine–pyrimidone dimer (6-4PP) and a DNA damage mimic at a variety of positions. We found that UV-DDB binds UV-damaged nucleosomes at lesions located in the solvent-facing minor groove without affecting the overall nucleosome architecture. For buried lesions facing the histone core, UV-DDB shifts the translational register of the DNA, moving the damage to an exposed position compatible with binding. These findings explain how UV-DDB detects lesions that are occluded in tightly positioned nucleosomes and details a nonenzymatic mechanism for high-affinity DNA-binding proteins, including transcription factors, to access nucleosomal DNA.

P034
Using deep phenotyping to refine the association of XPD (ERCC2) mutations with clinical disease

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XPD, coded by ERCC2 is a helicase component of the basal transcription factor, transcription factor II H (TFIIH), which is involved in both DNA repair and transcription. Different mutations in XPD cause radically different phenotypes, possibly reflecting the dual function of the protein. Xeroderma

pigmentosum (XP) may be associated with predominately repair-affecting mutations, whereas predominately transcription-affecting variants may cause trichothiodystrophy (TTD). XP is characterized by extreme sensitivity to UV radiation damage and sun-induced cancers. The characteristic symptom of TTD is brittle, sulphur-deficient hair, but subjects have a broad range of abnormalities including skeletal, developmental and immune. In addition, there is an intermediate phenotype (XP/TTD), sharing milder aspects of each disease. Both XP and TTD have a range of severity, suggesting a multidimensional gradation of phenotypes. We sought to obtain a more complete picture of the range of phenotypes and better predictors of the severity of disease by deep phenotyping of 66 patients (30 XP, 13 XP/TTD and 23 TTD) with two inherited mutations in XPD. We used data on more than 200 features, including characteristic symptoms (e.g. degree of sun sensitivity and hair structure), a wide range of additional disease features (hearing loss, skeletal abnormalities, measures of developmental delay) and standard clinical measurements. Previous studies have mapped mutations associated with XP and TTD onto the 3D structure of XPD, but there is not a simple dichotomy between these diseases. Our deep phenotyping data gives a continuous and multidimensional measure of phenotypic variation, with the primary axis distinguishing XP and TTD. Assigning individual variants to a specific disease is confounded by most subjects being compound heterozygotes; however, principal component analysis separates the effect of the individual's two mutations (provided one of the mutations occurs at least twice). We present the 51 mutations present in our subjects mapped onto the structure of XPD as determined by x-ray crystallography and freeze-fracture electron microscopy. Colouring the residues by the principal components of the phenotypes reveals a continuum of phenotypic information related to each mutation site.