8th WINTER RESEARCH CONFERENCES

Oxidative DNA Damage:
From Chemical Aspects to Biological Consequences

LES HOUCHES - France
Centre de l'Ecole de Physique

** 17 to 23 January 2009 **

PROGRAM
&
BOOK
OF
ABSTRACTS

Organized by the CERLIB (Club d'Etude des Radicaux Libres en Biologie) and the European COST network CM0603 "Free Radicals in Chemical Biology"

Foreword

The organizing committee of the 8th CERLIB Winter Conferences is pleased to welcome you in Les Houches. This meeting belongs to a series of scientific events related to the chemistry and biology of oxidative stress. However, the 2009 issue is special in two aspects. First, for the first time it is completely devoted to one major topic that focuses on DNA damage, in terms of formation, biological properties and consequences for human health. We are proud to have gathered some of the world's leaders in these fields.

A second motive of satisfaction is the fact that the European COST network CM0603 "Free Radicals in Chemical Biology" is co-involved in the organization of this joint meeting. The scientific activities performed by this group perfectly illustrate the advances that can be achieved by the combination of various and complementary expertise borrowed from chemistry and biochemistry. This overlapping between disciplines is also reflected in the whole program of the meeting as well as in the audience.

We would like to thank our sponsors who made this event possible. These include the Institut Nanoscience et Cryogénie of the Commissariat à l'Energie Atomique, the Joseph Fourier Grenoble University, the Institut National du Cancer, the EU COST programme and the Merck laboratories.

We hope that you will enjoy the scientific discussions, as well as the beauty of the Mont-Blanc mountain range and hopefully the various activities provided by the snow!

The organizing committee

Jean Breton, Jean Cadet, Serge Candéias, Thierry Douki, Alain Favier, Didier Gasparutto, Walid Rachidi, Jean-Luc Ravanat, Sylvie Sauvaigo, Zohra Termache

Program and Abstracts

COST Chemistry CM0603

Free Radicals in Chemical Biology

(CHEMBIORADICAL)

WP3 Meeting

January 17th & 18th 2009
Les Houches, France

Local organizer:

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Saturday, January 17

15h00: **Registration**

Sunday, January 18

08h20: **C. Chatgilialoglu** *Presentation of the COST Action CM0603 "Free Radicals in Chemical Biology"*

8h30-10h00: Cost session 1

08h30: **J.Cadet** *Oxidatively generated damage to DNA: recent insights and perspectives*

09h00: **C. Chatgilialoglu** *The chemistry of 5',8-cyclopurine lesions*

09:30: **L. Cellai** *Radiolysis of 8-bromo-2'-deoxyguanosine containing oligodeoxynucleotides*

10h00: Coffee break

10h30-12h00: Cost session 2

10h30: **M. Wolszczak** *Experimental studies of electron transfer within DNA duplex*

11h00: **M. Robert** *Proton-coupled electron transfer in bioradicals*

11h30 **T. Gimisis** *Independent photo-generation of one-electron oxidized guanine base: The complete story?*

15:00 WG discussions and planning

17h00-19h00: Cost session 3

17h00: **M. Miranda** *Use of nucleoside-drug conjugates as models to investigate the mechanisms of photosensitized DNA damage*

17h30: **T Carell** *The chemistry of DNA repair and DNA tolerance at atomic resolution*

18h00: **P. O'Neill** *Radiation-induced DNA double strand breaks – cleaning up the ends*

18h30: **T. Ozben** *Diverse effects of antioxidants on the cytotoxicity of chemotherapeutics*

Monday, January 19

8h30-10h00: Cost invited speakers

08h30: **B. Epe** *Oxidative base damage: biological relevance and repair*

09h15: **Z. Livneh** *Molecular mechanisms of error-prone and error-free translesion DNA synthesis in mammalian cells*

10h00: End of COST meeting, Coffee break

Oxidatively generated damage to DNA: Recent mechanistic insights and perspectives

Jean Cadet*[1,2], Thierry Douki[1], Didier Gasparutto[1], Jean-Luc Ravanat[1] and J. Richard Wagner[2]

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Major progress has been achieved during the last decade in the elucidation of oxidative degradation pathways of purine bases mediated by singlet oxygen ($^1O_2$), hydroxyl radical ($^{\bullet}OH$) and one-electron oxidants for which a general consensus now exists. Occurrence of nucleophilic addition reactions during the conversion of guanine radical intermediates into final decomposition products upon one-electron oxidation has allowed the proposal of two mechanisms for the formation of DNA-protein cross-links under aerobic conditions. Evidence has been provided in both cases for the formation of a covalent bond between the free ε amino group of a lysine residue and two different guanine transients at the C8 position. Another relevant example of decomposition pathway of the guanine radical cation is illustrated by the characterization of 2-deoxyribonolactone, an oxidized abasic site, as one of the main riboflavin photosensitized degradation products of a guanine containing 15-mer. The mechanism of specific deprotonation at C1' remains to be elucidated in relation with the recent proposal of photo-induced translocation of guanine radical to the 2-deoxyribose moiety of purine nucleotides in the solid state. The one-electron reduction of 8-hydroxy-7,8-dihydropurinyl radical that may be generated either by $^{\bullet}OH$ addition at C8 of the purine bases or hydration of related radical cations is known to lead to 2,6-diamino-4-hydroxy-5-formamidopyrimidine and 4,6-diamino-5-formamidopyrimidine (FapyAde) through the cleavage of the 8,9-imidazole bond. Interestingly the position isomer of FapyAde, namely 4,5-diamino-6-formamidopyrimidine that results from the opening of the imidazole ring between C8 and N7 has been found to be the main radiation-induced degradation product of adenine in oxygen-free aqueous solution in agreement with results of recent DFT calculations made on guanine. Another topic of major interest deals with the elucidation of radical oxidation mechanisms of cytosine and 5-methylcytosine in isolated DNA for which there is still a lack of information. This concerns in particular the rearrangement reactions following intramolecular cyclization of 6-hydroperoxy-5-hydroxy-5,6-dihydroderivatives of cytosine and 5-methylcytosine.

The chemistry of 5',8-cyclopurine lesions

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5',8-Cyclo-2'-deoxyadenosine and 5',8-cyclo-2'-deoxyguanosine are among the lesions formed in DNA. These modified nucleosides are the result of a rapid C5' radical attack to the purine moiety. Both derivatives can be formed in two diastereomeric forms depending on the configuration at C5' position. Structural information of the intermediate radicals and their fate, under anoxic or aerobic conditions as well as in the presence of thiols or metal ions, were obtained using nucleoside models. Efficient synthetic procedures based on one-pot radical cascade reactions have been developed.

Radiolysis of 8-bromo-2'-deoxyguanosine containing oligodeoxynucleotides

Luciano Cellai and Chryssostomos Chatgilialoglu

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The electron adduct of 8-bromo-2'-deoxyguanosine undergoes a fast protonation at C8 to afford the one-electron oxidized 2'-deoxyguanosine.[1,2] In particular, the two short-lived intermediates, which show substantial difference in their absorption spectra, were recognized to be the two guanyl tautomers. Product studies showed also that 8-bromoguanine derivatives are prone to capture e_{aq}^- with a quantitative formation of the corresponding debrominated nucleosides. Our attention is focused to the reactions of e_{aq}^- with a variety of single- and double-strand oligonucleotides containing 8-bromoguanine moieties. Our results confirmed that these bromo-derivatives are very effective in excess electron transfer and they can behave as an ultimate sink for electrons due to the Br^- ejection.[3] In order to define the reaction mechanism of these transformation, tailored experiments are in progress by radiolytic techniques.

1) Chatgilialoglu, C.; Caminal C.; Guerra, M.; Mulazzani, Q. G. Angew. Chem. Int. Ed. **2005**, 44, 6030–6032.

2) Chatgilialoglu, C.; Caminal C.; Altieri, A.; Mulazzani, Q. G.; Vougioukalakis, G. C.; Gimisis, T.; Guerra, M. J. Am. Chem. Soc. **2006**, 128, 13796–13805.

3) Manetto, A.; Breeger, S.; Chatgilialoglu, C.; Carell, T. Angew. Chem. Int. Ed. **2006**, 45, 318–321.

Experimental studies of electron transfer within DNA duplex

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Both experiment[1,2,3] and theory[4,5,6] have been applied to determine whether the array of π – stacked Watson-Crick base pairs facilitates charge transfer over extended molecular distances in the interior of the double helix. The process is important because charge migration through DNA plays a crucial role in mutagenesis and carcinogenesis. From a practical perspective, understanding of the charge migration process in DNA is important in the development of nanoscale electronic devices[6].

In this presentation we report on the pulse radiolysis studies under conditions where the electron is captured by DNA bases and subsequent electron transfer to the intercalator is observed. The main factors influencing such transfer will be analyzed, namely: the coupling of the intercalator into the base pairs stack and the driving force for electron scavenging reaction. The intercalator of high electron affinity: 1,4-dihydroxy-5,8-bis[[2-[(2-hydroxyethyl) amino]ethyl] amino]-9,10-anthracenodione (mitoxantrone) can induce long-range electron transfer restricted to less than nine base pairs.

To assess the role of the base pairs in mediating electron transfer we have studied fluorescence quenching of DNA bound intercalators (anthryl derivatives, ethidium bromide, ruthenium complexes) by the quenchers which have quite different interactions with DNA; i.e. other intercalators (e.g. amsacrine, ledakrin) and externally bound compounds (e.g. methylviologen). Special attention will be paid to the kinetic models for the description of the fluorescence quenching by electron transfer.

References:

1. C.R. Treadway, M.G. Hill, J.K. Barton, Chem. Phys., **2002**, 281, 409

2. A. Messer, K. Carpenter, K. Forzley, J. Buchanan, S. Yang, Y. Razskazovskii, Z. Cai, M. D. Sevilla, J. Phys. Chem. B, **2000**, 104, 1128

3. R.F. Anderson, K.B. Patel, W.R. Wilson, J. Chem. Soc. Faraday Trans., **1991**, 87, 3739

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Proton-coupled electron transfer in bioradicals

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Proton-coupled electron transfer reactions play a critical role on a wide range of natural and artificial processes. An important example is photosystem II, which converts water into oxygen thanks to the light energy. In this process, the oxidized chlorophyll P_{680}^{+} oxidizes a tyrosine: both electron and proton are transferred. Another facet of oxygen evolution is related to transition metal (Mn) aquo complex oxidation where proton transfer and electron transfer involve different molecular centers. One may wonder if the high efficiency of these reactions is related to the possibility that the electron and proton transfer steps are concerted. An other interesting example is given by DNA redox chemistry that may involve proton-coupled electron transfers, with potential impact on both charge migration and (radical) damage formation. Redox reactions and mechanisms involving model compounds will be discussed.

Use of nucleoside-drug conjugates as models to investigate the mechanisms of photosensitized DNA damage

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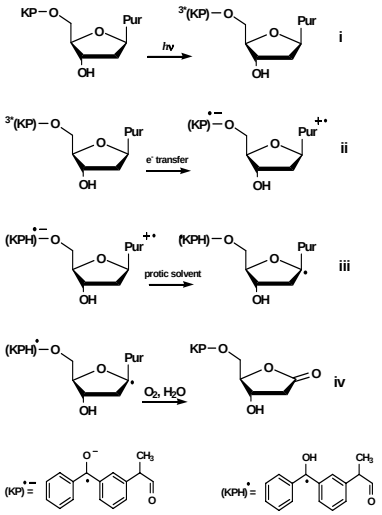

Formation of abasic sites in DNA may occur by generation of a C1' radical and subsequent formation of a 2-deoxyribonolactone (dL); abstraction of the H1' hydrogen at the anomeric carbon by an appropriate agent is the common initiation step. In the present work, several benzophenone (BP)-purine dyads have been submitted to a mechanistic study on the photosensitized oxidation reactions, with special emphasis on the possible formation of the dL lesion. Here the BP unit is provided by S-ketoprofen (KP), a well known photosensitizing drug, which has been previously employed for mechanistic studies on nucleosides.[1] The structural variations have been designed to evaluate the relative base reactivities (in the series adenine, 8-oxoadenine, guanine). Actually, a dL is formed by photolysis of the newly synthesized, KP-tethered purine nucleosides. Transient absorption spectroscopy supports an intramolecular electron-transfer mechanism and reveals that the process occurs from the short-lived BP-like triplet excited state, through generation of C1´-ketyl biradicals.

[1] (a) Lhiaubet-Vallet, V.; Encinas, S.; Miranda, M. A. J. Am. Chem. Soc. **2005**, 127, 12774–12775. (b) Encinas, S.; Belmadoui, N.; Climent, M. J.; Gil, S.; Miranda, M. A. Chem. Res. Toxicol. **2004**, 17, 857-862.

The Chemistry of DNA Repair and DNA Lesion Tolerance at Atomic Resolution

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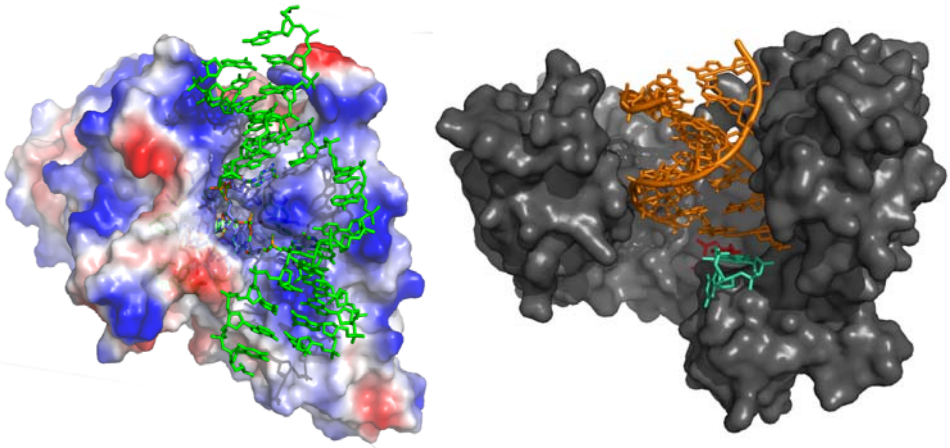
Our genome is constantly damaged by various exogenous and endogenous events. 20'000 to 40'000 lesions are in this way generated each day per cell. These lesions interfere with the normal transcription and replication events. In the lecture we will describe the chemical synthesis of oxidative DNA lesions, DNA lesions which are formed due to UV-irradiation, and of cisplatin lesions. We discuss how these lesions are incorporated into oligonucleotides using solid phase chemistry. Some of the obtained DNA strands, which contain a defined (6-4) lesion at a defined site, were used to create a co-crystal structures with the (6-4) DNA photolyase[1]. From this structure and correlated biochemistry we learn how nature achieves to repair UV induced lesions. Co-crystal structures of cisplatin lesion containing DNA in complex with polymerase-eta allows us to decipher the mechanism of translesion synthesis[2].



(6-4) DNA photolyase (left)[1] and polymerase-eta (right)[2] in complex with DNA containing lesions.

1. M. J. Maul, T. R. M. Barends, A. F. Glas, M. J. Cryle, T. Domratcheva, S. Schneider, I. Schlichting, T. Carell, *Angew. Chem. Int. Ed.* **2008**, *47*, 10076-10080. "Crystal Structure and Mechanism of a DNA (6-4) Photolyase

2. A. Alt, K. Lammens, C. Chiocchini, A. Lammens, J. C. Pieck, D. Kuch, K.-P. Hopfner, T. Carell, *Science* **2007**, *318*, 967-970. "Bypass of DNA lesions generated during anticancer treatment with cisplatin by DNA polymerase eta"

Radiation-induced DNA double strand breaks – Cleaning up the ends

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Complex double-strand breaks (DSBs) induced by ionising radiation and characterised by the presence of base lesions, AP sites or single-strand breaks close to the break termini, are believed to be one of the major causes of the biological effects of IR. It has been hypothesised that these complex DSBs pose problems to the DNA repair machinery. The major repair pathway of DSBs in mammalian cells is non-homologous end-joining (NHEJ) which involves the recruitment of the DNA-protein kinase complex which in turn recruits the DNA ligase IV/XRCC4 complex. However, the DSB termini may require processing prior to repair, particularly with complex DSBs, involving the base excision repair pathway (BER).

Double-stranded oligonucleotides were synthesised with 8-oxo-7, 8-dihydroguanine (8-oxoG) at defined positions relative to readily ligatable 3'–hydroxyl or 5'–phosphate termini. The break termini interfere with removal of 8-oxoG during base excision repair as elucidated from the severely reduced efficiency of 8-oxoG removal by OGG1 with AP endonuclease-1 when in close proximity to break termini. NEIL-1 however, can partially restore processing of complex DSBs in an AP endonuclease-1 independent manner. The influence of 8-oxoG on ligation shows delayed rejoining if 8-oxoG is positioned two to three bases from the 3'-hydroxy or six bases from the 5'-phosphate termini. When two 8-oxoG lesions are positioned across the break junction ligation is severely retarded. 3'-break ends are generally phosphates or phosphoglycolates (3'-PG) and require processing prior to ligation. 8-oxoG does not effect the efficiency of HAP-1 to remove 3'PG when present on 3'recessed or blunt ends of oligos, whereas a 3'PG overhang is not removed by HAP-1.

The reduced efficiency of repair indicates that complex DSBs are likely to persist longer than simple DSBs in cells, and as a consequence are more significant in contributing to the biological effects of ionising radiation. The role of glycosylases/endonucleases in orchestrating the processing of clustered sites will be discussed. Overall, the results demonstrate the importance of 'dirty' DSB to understanding the mechanisms of the biological consequences of DNA damage induced by ionising radiation.

We would also like to thank the Medical Research Council (UK) and the EU RISC-RAD project (contract FI6R-CT-2003-508842) for financial support.

Diverse effects of antioxidants on the cytotoxicity of chemotherapeutics

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There are conflicting views on the use of antioxidants in cancer patients, and their potential interactions with radiation and chemotherapy. Some chemotherapeutic agents and all radiation therapy induce oxidative stress by generation of oxygen free radicals (ROS) which might be an alternative mechanism for their cytotoxic effect via inducing apoptosis. A question has logically developed as to whether antioxidants taken concurrently during chemotherapy could reduce the beneficial effect of chemotherapy on malignant cells by inhibiting ROS and preventing apoptosis of cancer cells. In order to clarify the roles of antioxidants in chemotherapy, we investigated Quercetin (3,3',4',5,7-pentahydroxyflavone) and N-acetylcysteine (NAC) in different cell types treated with anticancer drugs. We studied cytotoxic activity of Topotecan alone and/or in combination with Quercetin in two human breast cancer cell lines, MCF-7 and MDA-MB-231. We also investigated the effect of NAC on MRP1-mediated doxorubicin and vincristine cytotoxicity in Human Embryonic Kidney (HEK293) and its MRP1 transfected (293MRP) cells. Our data indicated increased oxidative status in MCF-7 and MDA-MB-231 cells exposed to Topotecan. Treatment with Quercetin did not inhibit ROS generation, and enhanced cytotoxicity of Topotecan in both cells. In contrast, NAC enhanced resistance against doxorubicine and vincristine in MRP1 overexpressing cells. Our study demonstrates that Quercetin and NAC have diverse effects in the cytotoxicity of chemotheurapeutic drugs. We conclude that whether an antioxidant supplement would be helpful, harmful or neutral depends in part on the specific antioxidant (and its dose), the chemotherapy drugs being used, the type and stage of cancer being treated. Well designed randomized controlled trials are needed to fully elucidate the impact of single antioxidants and antioxidant combinations in conventional cancer therapy.

Oxidative DNA base damage: Biological relevance and repair

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Oxidative DNA base damage is endogenously generated in apparently all types of cells. Its adverse effects are partially prevented by specific repair mechanisms. In the case of 8-hydroxyguanine (8-oxoG), base excision repair, which in mammalian cells is initiated by the glycosylase OGG1, appears to be most important. However, a not fully elucidated mechanism depending on the activity of both CSB and PARP1 plays a role as well and serves as a back-up repair pathway. To evaluate the relevance of the endogenously generated 8-oxoG residues in the DNA for the initiation of carcinogenesis in man and animals, we have analysed the influence of genetic defects of *Ogg1* and *Csb* in mice on (a) the basal levels of 8-oxoG in the DNA of the livers of the animals (b) the spontaneous mutation rates and (c) the frequency of preneoplastic foci in the livers. The data indicate that relatively few additional endogenously generated oxidative DNA base modifications double the overall spontaneous mutation rates and give rise to additional liver tumours if liver cell proliferation is stimulated. Surprisingly, under conditions of elevated oxidative stress, which we generated in cultured cells by glutathione depletion and irradiation with visible light, the OGG1-mediated repair of oxidative base modifications is not accelerated, but rather retarded, while the repair of pyrimidine dimers, sites of base loss (AP sites) and single-strand breaks is not affected. Although the mechanism and reasons for the stress-dependent inactivation of OGG1 remain to be established, the data support the notion that the generation of endogenous oxidative DNA damage, particularly under conditions of oxidative stress, is an important risk factor for the initiation of carcinogenesis.

Molecular mechanisms of error-prone and error-free translesion DNA synthesis in mammalian cells

Zvi Livneh*, Sigal Shachar, Lior Izhar, Sheera Adar, Noam Diamant, Ayal Hendel, Omer Ziv, Hila Abutbul, Isadora Cohen and Zohar Goren

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Translesion DNA synthesis (TLS), also termed error-prone DNA repair, is a cellular DNA damage tolerance pathway, in which lesions causing replication blocks are overcome by specialized low-fidelity DNA polymerases. Due to the miscoding nature of most DNA lesions, this pathway is inherently error-prone. Mammalian cells contain 5 dedicated TLS DNA polymerases, and 5-10 additional polymerases that may participate in this process. TLS must be tightly regulated in order to avoid an escalation in mutations rates. This regulation is primarily at the posttranslational level, and involves monoubiquitination of PCNA, as well as the action of p53, and p21 via its interaction with PCNA. To elucidate the operation principles of TLS we used a quantitative assay for TLS that was developed in our lab, based on gapped plasmids carrying site-specific lesions, in conjunction with cultured mammalian cells in which the expression of specific TLS polymerases was either knocked-out or knocked-down. The results of these studies will be presented.

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CERLIB Meeting

Saturday, January 17

15h00: **Registration**

Monday, January 19

10h30-12h00: Pyrimidine Damage (*Chairman: Thierry Douki*)

10h30: **J. R. Wagner** *Free radical oxidation of cytosine and 5-methylcytosine: characterization of novel stable and unstable products*

11h00: **O. Brede** *Radical reactions in the system pyrimidine/aliphatic thiol*

11h20: **A. Banyasz** *Excited state relaxation and energy transfer in DNA double helices studied by femtosecond fluorescence spectroscopy*

11h40: **J. Wang** *A mechanistic study of the preferential photo-oxidation of the 5-CH_3 substituent of thymine and thymidine by $NH_4[VO(O_2)2(5\text{-}NO_2phen)]$*

12h00: Lunch & Free afternooon

17h00-18h50: Guanine Damage (*Chairman: Jean-Luc Ravanat*)

17h00: **C. J. Burrows** *Context effects on hydantoin formation and repair in DNA*

17h30: **N. E. Geacintov** *Reaction pathways of guanine radicals in solution and susceptibilities of removal of some of the stable reaction products by nucleotide excision repair mechanisms*

18h00: **G. Pratviel** *Guanine oxidation by high-valent metal-oxo porphyrins: electron transfer and oxygen atom transfer*

18h30: **V. Shafirovich** *Guanine lesions generated by oxyl radicals*

19h00: Welcome reception and dinner

Tuesday, January 20

8h30-9h50: Complex Lesions (*Chairman : Richard Wagner*)

8h30: **M. M. Greenberg** *Formation of DNA interstrand cross-links via oxidative stress*

9h00: **J.-L. Ravanat** *Complex DNA lesions generated subsequently to 2-deoxyribose oxidation*

9h30: **F. Bergeron** *Tandem lesions involving 8-oxo-purines in isolated DNA and polynucleotides: quantitation, mechanism of formation and repair*

10H40: Coffee break

10h20-12h00: Radiation-Induced DNA damage (*Chairman: Jean Cadet*)

10h20: **M. Sevilla** *Characterization of excitation induced hole transport and reactivity in DNA*

10h50: **C. Chatglialoglu** *Revising the reactions of hydroxyl radicals and hydrated electrons with guanine derivatives*

11h20: **M. Beuve** *Free radicals, oxygen effect and cell survival: an analysis by simulation*

11h40: **E. Dumont** *Chemical aspects of DNA damage: what can theoretical calculations tell us?*

12h00: Lunch & Free afternoon

17h00-19h10: DNA Lesions as Biomarkers (*Chairman: Alain Favier*)

17h00: **J. Cadet** *Oxidatively generated damage to DNA: measurement in cells*

17h30: **A. Collins** *Measurement of DNA damage and repair with the comet assay: recent developments*

18h00: **T. Douki** *HPLC-mass spectrometry analysis and Comet assay measurements of DNA damage induced by benzo[a]pyrene*

18h30: **M. Audebert** *Effect of Polycyclic Aromatic Hydrocarbons (PAHs) xenobiotics metabolism on DNA integrity, Development of a new genotoxic assay*

18h50: **S. Shaposhnikov** *Fluorescent in situ hybridisation on comets: Possible application to DNA damage and repair*

20h30: Poster Session

List of posters:

1. **F. Anquez & E. Courtade**
   Optical excitation and detection of singlet oxygen: Towards intracellular microscopy of reactive oxygen species
2. **A. Azqueta**
   Phytochemicals affecting DNA repair
3. **A. Boivin**
   The increase of oxidative stress after transient glutathione depletion and irradiation triggers apoptosis in head and neck squamous cell carcinoma
4. **C. Charles & C. Stevigny**
   Practicability of the Comet assay to investigate the influence of flavonoids on DNA repair kinetics
5. **M. Flaender**
   Pulsed EPR structural analyse of DNA damages caused by platinum anticancer agents
6. **T. Gehrke**
   Synthesis and incorporation of carbocyclic analogues of the oxidative DNA-Lesion FaPyG
7. **C. Bounaix Morand du Puch**
   Design of a ligand-fishing method for the characterization of proteins associated to DNA lesions generated by cisplatin
8. **E. Kaya**
   Identification of a new (6-4) photolyase
9. **S. Meier**
   Oxidative DNA damage induced by benzo(a)pyrene in the testis of Ogg1-deficient mice, and repair of such lesions in vivo
10. **B. Pons**
    Damaged-oligonucleotides biochip: a convenient means to assess the DNA repair ability of any biological sample
11. **M. Pitié**
    DNA cleavage by ditopic copper-platinum complexes
12. **D. Praseuth**
    Protein complexes assembled in vitro at double strand breaks
13. **A.-L. Raffin**
    Characterization of DNA repair phenotypes from XPA and XPC cell lines with an in vitro miniaturized test
14. **S. Sauvaigo**
    Response of cancer cell lines to genotoxic drugs in relation to chemoresistance: DNA repair phenotyping using a functional multiplex miniaturized assay
15. **R. Strasser**
    Rad14 confers specificity for bulky adducts in Nucleotide Excision repair
16. **V. Latypov**
    Base excision repair-induced single strand breaks initiate Rec12-independent meitoic recombination

Wednesday, January 21

8h30-9h50: Oligonucleotides for Biochemical Studies (*Chairman, Dimitar Anguelov*)

8h30:	**S. Gambarelli**	*Probing lesion-induced DNA conformational changes by pulsed EPR*
9h00:	**Y. Wang**	*Chemistry and biology of complex lesions induced by reactive oxygen species*
9h30:	**M. Lomax**	*Processing of clustered DNA damaged sites comprised of three lesions by the base excision repair pathway*

9h50 : Coffee break

10h20-12h00: DNA Repair (1) (*Chairman: Alain Sarazin*)

10h20:	**D. Angelov**	*Base excision repair within nucleosomal substrates*
10h50:	**M. Atta**	*DNA repair and free radicals: New insights into the mechanism of spore photoproduct lyase revealed by single mutation*
11h20:	**J. Timmins**	*Structure-function studies of DNA repair proteins from Deinococcus radiodurans*
11h40:	**A.-S. Belmont**	*Monitoring small molecules impact on cell repair capability using lesion containing oligonucleotide microarrays*

12h00: Lunch & Free afternoon

17h00-18h50: DNA Repair (2) (*Chairman: Peter O'Neill*)

17h00:	**L. Mullenders**	*Repair and biological effects of spontaneous and ionizing radiation induced DNA damage*
17h30:	**A. Sarasin**	*Transcription-coupled repair-deficient diseases: From Cockayne syndrome to UVSS syndrome*
18h00:	**S. Sauvaigo**	*Determination of the effects of aging and photoaging on DNA repair excision/synthesis capacities toward different DNA lesions using a functional biochip assay*
18h30:	**A. Khobta**	*Base excision repair protein Ogg1 contributes to impaired transcription at the sites of oxidative base damage*

20h30: Round Table Discussion "Tools for DNA Repair Quantification"

Organized by **S. Sauvaigo**

Thursday, January 22

8h30-9h50: DNA Polymerases (*Chairman*: *Evelyne Sage*)

8h30:	**R. Fuchs**	*Role of an Alkyltransferase-like protein in E. coli: the ybaZ gene product enhances Nucleotide Excision Repair of O6-alkylguanine adducts*
9h00:	**C.-A. Reynaud**	*Error-prone repair pathways in immunoglobulin gene hypermutation*
9h30:	**G. Henneke**	*Oxidative lesions processing by high-fidelity DNA polymerases in Pyrococcus abyssi*

9h40 : Coffee break

10h20-12h00: Cellular effects of oxidative stress to DNA (*Chairman Robert Fuchs*)

10h20:	**E. Sage**	*Repair of oxidized bases within multiply DNA damaged sites and mutation formation*
10h50:	**Y. Saintigny**	*Genetic Instability induced by tritium contamination*
11h20:	**A. deGroot**	*Deinococcus deserti: radiation tolerance and adaptation to harsh environmental conditions*
11h40:	**V. De Rosa**	*The anticancer effect of selenium: study of its role in DNA repair activity and resistance to UVA*

12h00: Lunch & Free afternoon

17h00-18h50: Oxidative DNA Damage and Human Diseases (*Chairman: Serge Candéias*)

17h00:	**A. Favier**	*Oxidative stress, DNA damage and male infertility*
17h30:	**L. Hardie**	*Oxidative DNA Damage in Barrett's Oesophagus*
18h00:	**R. Olinski**	*Possible link between oxidative DNA damage and cancer: i/ case of BRCA1 mutation carriers, ii/ relationship between DNA oxidation and methylation*
18h30:	**T. Paz-Elizur**	*Repair of oxidative DNA damage and the risk of tobacco-related cancers*

Friday, January 23

Breakfast and safe return home!

Abstracts

Oral presentations

Free radical oxidation of cytosine and 5-methylcytosine: Characterization of novel stable and unstable products

J. Richard Wagner[1], Nourreddine Belmadoui[1] and Jean Cadet[1,2]

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Oxidative damage to cytosine in DNA has a high probability of causing mutations as inferred by the bias toward CG to TA transitions in the spectra of spontaneous and oxidant induced mutations. The majority of studies to date have focused on 3 stable oxidation products of cytosine, which includes 5-hydroxycytosine, 5-hydroxyuracil and uracil 5,6-glycols. However, there is a multitude of other stable and unstable products of cytosine that may have a considerable impact on DNA repair and potential mutagenesis in cellular DNA. Recently, we identified several stable and unstable products involved in the hydroxyl radical and one-electron oxidation of 2'-deoxycytidine. The stability of cytosine 5,6-glycols was examined in the free nucleoside, and oxidized calf thymus DNA and poly(dG-dC). The secondary structure of DNA appears to greatly stabilize cytosine 5,6-glycols and increase the extent of deamination in comparison to the modified nucleoside. The chemistry of several unstable products of cytosine was also investigated by mild oxidation of 5-hydroxycytosine in the nucleoside and oligonucleotides. This reaction results in a cascade of decomposition reactions involving the formation of isodialuric acid, dialuric acid, hydroxyhydantoin and isohydroxyhydantoin products Lastly, a unique pathway of cytosine and 5-methylcytosine oxidation is depicted by the rearrangement of intermediate 5(6)-hydroxy-6(5)-hydroperoxide into novel imidazolidine 4,5-glycols and related products. These studies provide further insight into the potential deleterious effects of cytosine oxidation in cellular DNA.

Radical reactions in the system pyrimidine/aliphatic thiol

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Aliphatic thiols are very efficient antioxidants in particular in biological systems. In this paper we focus on two modes of thiol action such as (i) the rate of the repair of pyrimidine-derived radicals by natural appearing thiols and (ii) the interaction of aliphatic thiols with the pyrimidine C5-C6 double bond.

(i) Although the repair of C-centered radicals by thiols is a well known process, the exact rate of the elementary reaction (1) could not be determined up to now. The reason for it is the high reactivity of the thiols with any present radicals.

$$Pyr^{\bullet} + RSH \rightarrow PyrH + RS^{\bullet} \qquad (1)$$

We generated pyrimidinyl radicals of various structures ($Pyr^{\bullet}$) in aqueous and alcohol containing solutions by means of pulse radiolysis in order to determine the rate constants of their repair reactions (1) by different thiols (RSH=cysteamine, 2-mercaptoethanol, cysteine and penicillamine) and found a way for dissolving the problem of the very complex competition between the existing radical reactions [1]. Hence we can state that C5-OH and C6-OH adduct radicals of the pyrimidines react with thiols with $k_1=(1.2\text{-}10.0) \times 10^6$ dm^3 mol^{-1} s^{-1}. Similar repair rate constants were found for uracil and thymine derived N1-centred radicals, $k_1=(1.5\text{-}6.1) \times 10^6$ dm^3 mol^{-1} s^{-1}. However, pyrimidine radical anions protonated at their C6 position and C6-uracilyl radicals, with carbonyl groups at their C5 position, react with thiols faster, with $k_1=(0.5\text{-}7.6) \times 10^7$ dm^3 mol^{-1} s^{-1} and $k_1=(1.4\text{-}4.8) \times 10^7$ dm^3 mol^{-1} s^{-1}, respectively.

(ii) Furthermore, we found an interaction of thiyl radicals with the pyrimidine C5-C6 double bond which was unknown till now [2]. Addition and elimination interaction of thiyl radicals with the C5-C6 double bond in pyrimidines (2,-2) was studied by the pulse radiolysis technique in aqueous solution with the use of different monitoring systems.

$$Pyr + RS^{\bullet} \rightleftharpoons Pyr^{\bullet}(RS) \qquad (2,-2)$$

For this purpose *p*-thiocresol, cysteamine hydrochloride and mercaptoethanol were used. The rate constants of addition and elimination of thiyl radicals were determined by applying the modified version of ACUCHEM (Computer Program for Modelling Complex Reaction Systems)[3]. Aliphatic thiyl radicals add to pyrimidine C5-C6 double bond with $k_2=1.0\text{-}3.0\times10^7$ dm^3 mol^{-1} s^{-1}, whereas elimination takes place with $k_{-2} = 0.7\text{-}2.0\times10^5$ s^{-1}. Quantum chemical calculations at the B3LYP/6-31G(d)/PCM level show that the addition should occur at the C6-position of the pyrimidine ring and that the energy of interaction between thiyl radicals and pyrimidine double bond C5-C6 is relatively low.

[1] A. Wójcik,S. Naumov, B. Marciniak, O. Brede, *J .Phys. Chem. B* **110** (2006) 11906

[2] A.Wójcik,S.Naumov,B.Marciniak,R.Hermann,O.Brede, *J.Phys.Chem. B* **109**(2005)15135

[3] W. Braun, J.T. Herron, D.K. Kahaner, *Int. J. Chem. Kin.* **20** (1988) 51

Excited state relaxation and energy transfer in DNA double helices studied by femtosecond fluorescence spectroscopy

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Direct absorption of UV radiation by DNA bases is known to trigger photochemical reactions which may lead to carcinogenic mutations. Although the major lesions are well characterized, the fundamental processes preceding their formation are not yet understood. In order to shed some light on these aspects we studied the exited states and energy transfer in model DNA double helices, composed of either adenine-thymine (A-T) or guanine-cytosine (G-C) base pair using fluorescence upconversion. This spectroscopic technique allows the recording of fluorescence decays, fluorescence anisotropy decays and time-resolved fluorescence spectra with femtosecond resolution. The time-resolved data combined with steady-state spectra lead to the following picture. The Franck-Condon excited states of DNA helices cannot be considered as the sum of their monomeric constituents because electronic coupling induces delocalization of the excitation over a few bases. Energy transfer takes place via intraband scattering in less than 100 fs. The fluorescence lifetimes of DNA helices corresponding to $\pi\pi^*$ states are in general longer than that of an equimolar mixture of the corresponding nucleotides; a striking exception is observed for alternating G-C polymers.

A mechanistic study of the preferential photo-oxidation of the 5-CH_3 substituent of thymine and thymidine by $NH_4[VO(O_2)_2(5\text{-}NO_2phen)]$

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The photo-oxidation product profiles of thymine (T) and thymidine (dT) mediated by the $NH_4[VO(O_2)_2(5\text{-}NO_2phen)]$ complex ($VPNO_2$, where 5-NO_2phen = 5-nitro-1,10-phenanthroline) were characterized and quantified using HPLC, GC-MS, NMR, ESI-MS and compared to those obtained using 2-methyl-1,4-naphthoquinone (MQ) as the photo-oxidant. A preferential oxidation of the 5-CH_3 substituent over the commonly observed oxidation of the electron-rich C5-C6 double bond of thymine was observed in the $VPNO_2$-mediated reaction, affording, for example, 74% of 5-formyl-2'-deoxyuridine *vs*. 13% of thymidine glycol from the photo-oxidation of dT. Thymine radical cation, $[T\bullet]^+$, and thymine allyl radical, formed from 5-CH_3 deprotonation of $[T\bullet]^+$, were shown to be the key intermediates in this reaction by transient absorption spectroscopy and EPR spin trapping technique, respectively. The observed strong preference towards 5-CH_3 oxidation was interpreted to be the result of the combination of two events: (i) the π-stacking interaction between thymine and the 1,10-phenanthroline ligand of $VPNO_2$ (K_{bind} ~ 10 M^{-1} from ^1H NMR titration) and more importantly, (ii) one of the 5-CH_3 hydrogens of T was seen to position within H-bonding distance (d = 2.563 Å, from DFT calculation) to one of the NO_2-oxygens of $VPNO_2$, which presumably facilitated the 5-CH_3 deprotonation of the thymine radical cation generated from a primary one-electron transfer step, leading to the 5-CHO product.

Context effects on hydantoin formation and repair in DNA

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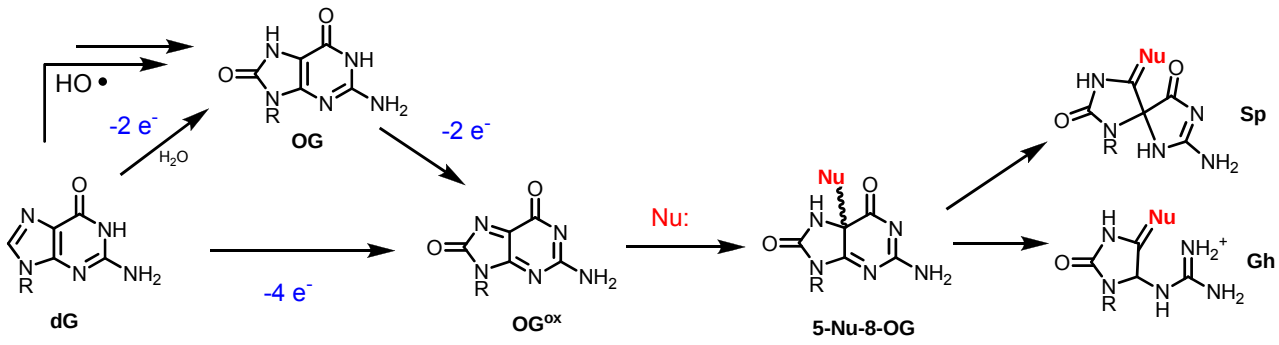
The common DNA oxidation product 8-oxoguanosine is highly sensitive to further oxidation leading to a family of hydantoin lesions including spiroiminodihydantoin (Sp) and guanindinohydantoin (Gh). In the presences of nucleophiles besides water, analogous adducts are formed in which one of the oxo moieties of Sp or Gh is replaced by nucleophiles such as spermine and lysine. Both the reactivity and the product distribution of 8-oxoG oxidation are influenced by the local context of the lesion. In addition, the ability of DNA repair enzymes such as hNEIL1 to recognize and excise the Sp and Gh substrates is dependent on the base opposite the lesion as well as the strand structure; results for ssDNA, dsDNA, bubble and bulge structures will be presented.



Reaction pathways of guanine radicals in solution and susceptibilities of removal of some of the stable reaction products by nucleotide excision repair mechanisms

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In cellular environments, oxidative stress is associated with the overproduction of nitric oxide, superoxide radical anions, and peroxynitrite. The major mode of reaction of peroxynitrite is believed to be the formation of a nitrosoperoxycarbonate complex with CO_2, that is followed by its rapid decomposition to the oxidizing $CO_3^{\bullet-}$ and $^{\bullet}NO_2$ radicals. Only the $CO_3^{\bullet-}$ radical can directly abstract an electron from guanine, the most easily oxidizable normal DNA base. This one-electron transfer step generates the guanine radical cation, $G^{+}\bullet$, and its deprotonated form, the neutral guanine radical, G(-H)•. We have extensively studied the reaction pathways of guanine radicals in solution under different reaction conditions. For example, the further reaction of $CO_3^{\bullet-}$ with G(-H)• (or 8-oxoguanine) in DNA yields the stereoisomeric spiroiminodihydantoin (Sp) and other lesions. The reaction of $^{\bullet}NO_2$ with G(-H)• radicals yields 5-guanidino-4-nitroimidazole (NIm) and the unstable product 8-nitroguanine. We recently identified the sequence-dependent reaction of G(-H)• radicals in DNA with an adjacent thymine or a next-nearest neighbor T to form the intrastrand cross-linked products …C8-G*-N3-T*… (G*T*) and …C8-G*-C-N3-T*… (G*CT*), respectively (Crean et. al., *Nucl. Acids. Res.* 36:742, 2008). Although neither of these lesions are bulky, both the Sp and G*-T* lesions cause local distortions in the double-helical DNA structure. It was therefore suspected that these lesions may also be good substrates for repair by the human Nucleotide Excision Repair (NER) mechanism that normally removes bulky lesions from genomic DNA, thus preventing mutations. Both Sp lesions, as well as G*CT* positioned in double-stranded DNA were medium to excellent substrates of NER in cell-free extracts from human HeLa cells, while the G*T* cross-linked product was only weakly incised. In contrast the NIm lesion was resistant to NER by the human DNA repair apparatus. In the case of the Sp lesions, but not the intrastrand cross-linked G*T* and G*CT* lesions, base excision repair is also active in human cell extracts. The presence of structural distortions rather than the presence of a bulky lesion appears to be sufficient for eliciting the efficient recognition and excision of such lesions by human NER factors. Although the Sp lesions are excellent substrates for Base Excision Repair, the NER system may function as an efficient backup in human cells.

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Guanine oxidation by high-valent metal-oxo porphyrins: Electron transfer and oxygen atom transfer

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Guanine oxidation products are most of the time products resulting from multiple events of oxidation. The most famous example is the first oxidation of guanine into 8-oxo-7,8-dihydroguanine (8-oxo-G), which is then oxidized into secondary oxidation products. This reaction pathway is typical of one-electron transfer reactions for which guanine oxidation starts with modification at the C8 carbon of G. We will present new results on the mechanism of guanine oxidation mediated by high-valent metal-oxo porphyrins, two-electron abstracting reagents. In that case, guanine modification first occurs at the C5 carbon. Further oxidation events are not due to electron transfer processes. They correspond to oxygen atom transfer reactions.

Guanine lesions generated by oxyl radicals

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Inflammation is tightly associated with malignant cell transformation and development of many human cancers. Persistent oxidative stress developed in response to inflammation in tissues results in overproduction of oxyl radicals and enhanced lipid peroxidation. Among the oxyl radicals that may be produced at sites of inflammation carbonate radical anions can selectively oxidize guanine in DNA by one-electron abstraction mechanisms and the guanine radicals thus formed decay by competing pathways with other free radicals and nucleophiles to form diverse guanine lesions. In turn, activation of lipid hydroperoxides via one-electron reduction produces electrophilic alkoxyl radicals that can damage cellular DNA by hydrogen atom or electron abstraction mechanisms. We have devised photochemical methods for the selective generation of oxyl radicals and explored the end products of their reactions with DNA by LC-MS/MS methods. One-electron abstraction from guanine in 5'-GCT sequences by carbonate radicals triggers the formation of diverse guanine lesions including 8-oxoguanine, guanidinohydantoin, spiroiminodihydantoin and a new type of intrastrand cross-linked product between guanine and thymine. These cross-links were excised from the oxidized oligonucleotides by enzymatic digestion with nuclease P1 and alkaline phosphatase and identified by LC-MS/MS, 1D and 2D NMR methods as a dG*-dT* dimer in which in which the guanine C8-atom is bound to the thymine N3-atom. In turn, combination reactions of guanine radicals with radical species produced by the one-electron reduction of lipid hydroperoxides such as 13*S*-hydroperoxy-9*Z*,11*E*-octadecadienoic acid (13*S*-HPODE) induce the formation of guanine alkylation products. In deoxygenated solutions we isolated two novel guanine adducts with molecular masses larger by 70 Da units than the parent sequences. The modified guanines were excised from the oligonucleotide adducts by enzymatic digestion with nuclease P1 and calf intestinal phosphatase, and identified by LC-MS/MS as the 8-pentyl- and N^2-pentyl-2'-deoxyguanosine.

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Formation of DNA interstrand cross-links via oxidative stress

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Oxidative nucleic acid damage is a complicated process that significantly affects the biopolymers' structure and function. Organic chemistry is a powerful tool that greatly facilitates biochemical studies on these processes. Our research group has used organic chemistry to independently generate reactive intermediates produced as a result of oxidative stress at defined sites within DNA and elucidated the chemistry of these species. In one instance, we have discovered a pathway by which a DNA radical forms interstrand cross-links by reacting with an opposing nucleotide. The discovery and subsequent application of this process will be discussed.

Complex DNA lesions generated subsequently to 2-deoxyribose oxidation

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Radiation-induced formation of DNA lesions has been studied in details during the last three decades and about 70 DNA lesions have been identified so far. However, works have mostly focused on DNA bases and sugar oxidation reactions are known to produce strand breaks that could be rapidly and efficiently repaired by the cellular machinery.

Recently, using a new strategy (Regulus et al. 2004) we have detected four new DNA lesions generated upon treatment of aerated aqueous solution of isolated DNA by gamma irradiation. Among them one was of particular interest since we have demonstrated that this lesion could be considered as a clustered DNA damage

The mechanism of formation of the detected clustered lesion involves first hydrogen abstraction at the C4' position that produces a modified sugar residue exhibiting a reactive α,β-unsaturated aldehyde (Regulus et al. 2007). Such an electrophilic compound then reacts with a proximate nucleophilic cytosine base by a mechanism similar to that described for 1,4-dioxo-2-butene to produce cytosine adducts identified as the four isomers of 6-(2-deoxy-ß-D-erythro-pentofuranosyl)-2-hydroxy-3(3-hydroxy-2-oxopropyl)-2,6-dihydroimidazo[1,2-c]-pyrimidin-5(3H)-one.

Thus formation of the lesions involves a strand break together with a cross-link between the modified sugar residue and a cytosine base, most probably located on the complementary strand. Other DNA adducts produced subsequently to 2'-deoxyribose oxidation have been also described in the literature (Dedon 2008) indicating that sugar oxidation could produce more complex DNA lesions than efficiently repaired single strand breaks.

Our work demonstrates that a single radical event could induce the formation of a clustered DNA lesion within cellular DNA. The difficulty of repairing such DNA lesions may explain, at least partly, in addition to double strand breaks formation, the origin of bleomycin and ionizing-radiation toxicity.

References:

Regulus, P., Spessotto, S., Gateau, M., Cadet, J., Favier, A. and Ravanat, J.-L. (2004) Detection of new radiation-induced DNA lesions by liquid chromatography coupled to tandem mass spectrometry. *Rapid. Commun. Mass Spectrom*., **18**, 2223-2228.

Regulus, P., Duroux, B., Bayle, P.-A., Favier, A., Cadet, J. and Ravanat, J.-L. (2007) Oxidation of the sugar moiety of DNA by ionizing radiation or bleomycin could induce the formation of a cluster DNA lesion. *Proc. Nat. Acad. Sci., U.S.A.*, **104**, 14032-14037.

Dedon, P.C. (2008) The chemical toxicology of 2-deoxyribose oxidation in DNA. *Chem. Res. Toxicol.*, **21**, 206-219.

Tandem lesions involving 8-oxo-purines in isolated DNA and polynucleotides: Quantitation, mechanism of formation and repair

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Complex DNA lesions are constituted of several DNA modifications localized within one or two DNA helix turns. This specific arrangement, that includes strand breaks, several single lesions or a combination of the two latter degenerated species, represents a challenge for the DNA repair systems in cell. Among these complex lesions, tandem lesions may arise from a single radical event, which leads to the formation of two neighbouring lesions on the same DNA strand. Using a simple analytical method based on the use of HPLC coupled to tandem mass spectrometry detection, we demonstrate that the ratio of 8-oxodGuo involved in a tandem lesion was about 50% of the total 8-oxo-dGuo generated upon gamma irradiation of aqueous aerated solution of DNA. We show that the mechanism of formation of 8-oxo-dGuo involved in tandem lesions involves first HO° addition onto a neighbouring base (most probably a pyrimidine). The generated radical reacts with molecular oxygen to produce a peroxyl radical that subsequently add to the C8 position of the vicinal purine. Decomposition of the unstable endoperoxide produces 8-oxodGuo[1]. Interestingly, a similar mechanism is also involved in the formation of 8-oxodAdo in tandem lesions.

Altogether, these results point out the crucial role of the three-dimensional DNA structure during the formation of lesions, and the unexpected high ratio of formation of tandem lesions in isolated DNA. The formation mechanisms of the tandem lesions in duplex DNA and polynucleotides will be discussed, and preliminary lesions repair assays will be presented.

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Characterization of excitation induced hole transport and reactivity in DNA

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In our recent efforts we have investigated the hole transport and reactions within DNA. We have investigated the effect of visible excitation on guanine and adenine cation radicals in model compounds and in DNA and RNA oligos.[1] Excited states of DNA bases are known to quickly deactivate and therefore, have been thought to have little biological significance in the radiation-induced damage. However, when combined with holes our recent work has shown that excited states of DNA holes directly result in DNA sugar radicals.[1] We found that the guanine cation radical excited state ($G^{\bullet +*}$) in DNA oligos produces C1'• and C5'• whereas in the nucleoside (2'-dG) we find C1'•, C3'• and C5'•. These sugar radicals result from deprotonation of the sugar ring cation radical. Our studies showed while C5'• and C1'• are formed in short oligos as the oligos increase in length, the C1'• increasingly dominates until in salmon sperm DNA only C1'• is found. In addition, we find that long visible wavelengths become ineffective in radical production as the strand increases in length. This is a result of base-to-base hole transfer which competes with base-to-sugar hole transfer a finding confirmed by theory (TD-DFT).[2] Recent work has elucidated the site of hole localization at G in G stacks in ds DNA-oligomers as well as the state protonation of G•+ in ss and ds DNA-oligomers. Our work has shown that holes in adenine stacks are stabilized by charge resonance interactions leading to delocalization between adjacent adenine bases and a likely explanation for the facile transfer of holes through A stacks found by a number of workers.[3] Finally we note that we have tested and confirmed the hypothesis that in gamma irradiated DNA long range hole transfer localizes multiple oxidative damages to a single guanine base resulting in an 8-oxoG$^{\bullet +}$ radical.[4] In order for this radical to form, three one-electron oxidations and a hydration reaction must occur at one guanine. This mechanism provides a means of self-radioprotection of DNA as it takes several potentially damaging DNA intermediates and produces a single repairable lesion on DNA.

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L. I. Shukla, A. Adhikary, R. Pazdro, D. Becker and M.D. Sevilla, Nucleic Acids Research, 2004, 32, 6565-6574.*

Revising the reactions of hydroxyl radicals and hydrated electrons with guanine derivatives

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The main reaction of the hydroxyl radical with guanine moiety is believed to be the addition at the C4 position with formation of an adduct, which undergoes a dehydration reaction to give the guanyl radical. On the other hand, the reaction of hydrated electrons with guanine moiety affords the electron adducts that after fast protonation at a heteroatom, is suggested to undergo a rapid transformation with formation of H atom adduct at C8. Our pulse radiolysis reinvestigation of these reactions showed that the tautomerization is a general phenomenon of the guanine-derived reactive intermediates.

Free radicals, oxygen effect and cell survival: an analyze by the simulation

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Cell killing by low Linear-Energy-Transfer (LET) radiations increases with tissue oxygenation. However this oxygen effect vanishes with increasing LET. Various scenarios have been proposed to explain the origin of the oxygen effect (formation of peroxidation cascades, fixation / restoration of DNA damage…) and its reduction at high LET (oxygen-in-the-track, interacting radicals…). With the aim of clarifying/quantifying some of them, we present a complete simulation of the production of free radicals by liquid-water radiolysis. We simulated the irradiation by X-rays and by fast ions with a LET ranging from 1 to 300 keV/µm.

Water radiolysis is considered as the succession of three stages. The physical stage includes the excitation and the ionization of water molecules by the incident ion and the subsequent secondary-electron cascades. The physico-chemical stage simulates the fast dissociation and rearrangement processes following immediately the excitation or ionisation of a water molecule in the liquid. Then the chemical stage accounts for diffusion and chemical reactions of these chemical species during the first 10 microseconds following the incident particle interaction.

Our simulation takes into account explicitly the multiple-ionisation process responsible for the production of oxygen in the track core (oxygen-in-the-track). We simulated the chemical species generated in the track and analyzed their spatial distributions. We also introduced in our simulation oxygen quantities ranging from hypoxic conditions to 1 bar pressure and found interesting correlations between the oxygen effect and the production of HO_2/O_2^-.

Chemical aspects of DNA damage: What can theoretical calculations tell us?

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DNA damage covers a vertiginous ensemble of chemical reactions, and is a recent area of research for theoretical approaches as they may provide complementary insights. An accurate multi-layer description is mandatory for macromolecular systems: cleavage and formation of chemical bonds description belong to quantum mechanics (QM) methods, while the rest of the system is treated at a molecular mechanics (MM), or further at a coarse-grain level. In this talk, the principle of hybrid QM/MM approaches will be explained. The definition of a frontier bond, denoted as '/', is specifically delicate for DNA; we have developed the use of frozen orbitals to achieve the most natural partition. [1] This way, systems of increasing complexity are considered on the same footing. A bottom-up approach might be a safer option towards a sound understanding of factors leading to the (often drastic) modulation of reaction pathways as the nucleotide of interest is inserted in an helix. Results are discussed for low-energy electrons addition on DNA. Far from being only a fictitious prototype reaction, experimental studies [2] have ascertained their key role in inducing DNA single-strand breaks (SSB). They lie at the heart of a rich and highly competitive chemistry, and state-of-the-art theoretical investigations on isolated nucleotides have shed lights on possible mechanisms. [3] We have evidenced huge variations of electron affinities and activation barriers (by a factor of 3) for cytosine or guanine considered in short oligonucleotides. Their origin (electrostatic, geometrical...) can be traced back by simple decomposition.

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Oxidatively generated damage to DNA: measurement in cells

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The determination of oxidative degradation pathways of nucleobases and 2-deoxyribose in cellular DNA has been hampered during more than two decades by the occurrence of major drawbacks that were associated with the available assays aimed at measuring damage. This which was due to self-radiolysis processes in the method developed initially to measure 5,6-dihydroxy-5,6-dihydrothymine and by spurious oxidation of overwhelming normal bases during the derivatization step of the gas chromatography-mass spectrometry assay has led to overestimation of the yields of oxidatively generated base and sugar lesions by factors varying between two and four orders of magnitude. More recently and thanks in particular to the cooperative efforts of the “European Standard Committee on Oxidative DNA Damage”, an European network devoted to the improvement and optimization of chromatographic and biochemical methods for measuring 8-oxo-7,8-dihydroguanine, significant progress has been made in the assessment of the latter oxidized base and several other base damage in cellular DNA. This was achieved using optimized conditions of DNA extraction and accurate analytical techniques including HPLC associated with either the electrochemical detection or the more versatile tandem-mass spectrometry technique. Thus, the measurement of more than 18 modified nucleosides and bases using the latter HPLC-MS/MS in the accurate and sensitive multiple reaction monitoring mode in association with the dilution isotopic technique has allowed the elucidation of the mechanism of oxidation reactions of bases in cellular DNA upon exposure to $^{\bullet}$OH, one-electron oxidants and singlet oxygen. The obtained data indicate that the levels of single base damage under acute conditions of exposure to oxidizing agents is at highest of a few lesions per 10^6 bases and that a good analogy exists with the results from model studies performed in aqueous solutions. However there are major divergences with the data that were obtained using the questionable HPLC-MS when high sensitivity is required. This concerns in particular the formation of radiation-induced purine 5’,8-cyclonucleosides which appears to be overestimated at least by 3 orders of magnitude with respect to HPLC-MS/MS measurements.

Measurement of DNA damage and repair with the comet assay: Recent developments

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Various methods are in common use to measure DNA oxidation. The ESCODD trials showed that, while HPLC-ECD is able to detect relatively high levels of 8-oxoGua induced experimentally in cellular DNA, it is affected by oxidation occurring during sample preparation, so that levels of background damage tend to be over-estimated. In contrast, the comet assay (in combination with formamidopyrimidine DNA glycosylase, FPG, to convert 8-oxoGua to DNA breaks) detects low levels of damage with accuracy if not precision. The flow of reports of 8-oxoGua concentrations well above the credible level has not abated since ESCODD conclusions were published.

The level of DNA oxidation is kept low by efficient DNA repair. The comet assay can be applied in two ways to measuring this repair. First, damage can be induced in cellular DNA, and the residual damage measured at intervals during incubation. For example, using FPG, base excision repair of 8-oxoGua (induced by treating cells with a photosensitiser plus light) can be followed. The alternative approach is the in vitro assay, in which a whole-cell extract is incubated with substrate nucleoids containing specific damage (e.g. 8-oxoGua), and accumulation of breaks – the initial stage of base excision repair – is measured during a short incubation. The assay has recently been modified to measure nucleotide excision repair.

DNA repair capacities vary widely among healthy individuals. The influences of intrinsic and extrinsic factors in determining repair capacity are far from clear. However, there is increasing evidence that repair is modulated by nutritional factors. Studies of genetic polymorphisms in DNA repair genes indicate that there may be a contribution of nucleotide excision repair to the repair of oxidation damage.

HPLC-mass spectrometry analysis and Comet assay measurements of DNA damage induced by benzo[a]pyrene

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Polycyclic aromatic hydrocarbons (PAH) produced upon incomplete combustion of organic matter are of concern to human health since some of them have been shown or are suspected to be carcinogenic to humans. The extent of DNA damage in populations exposed to PAH, and especially in mixtures of such compounds, is thus a major issue and requires reliable tools. The DNA lesions resulting from exposure to HAP result from the addition of reactive metabolites to DNA and from oxidative damage induced by the oxidative stress associated with metabolization. In the present work, we especially studied the genotoxicity of benzo[a]pyrene (B[a]P), the only HAP classified as recognized carcinogen to human. A comparison was made between the measure of DNA strand breaks and alkali-sensitive sites as determined by the Comet assay, and the HPLC-mass spectrometry quantification of DNA adducts to the diol epoxide derivative of B[a]P, its most reactive metabolite. For this purpose, HepG2 cultured human hepatocytes were treated with either pure B[a]P or particulate mixture collected in an urban peri-industrial site or in a metallurgic plant. Treatment with pure B[a]P did not induce increase in Comet measurements below a concentration of 1 µM whereas adducts were observed for concentrations as low as 0.025 µM. Very different results were observed with environmental samples. Increase in the Comet score was observed with both urban and industrial samples containing 0.16 µM (40ng/ml) of B[a]P, especially for samples of urban origin. In addition, a 30 % potentialization and a 90 % inhibition in the level of DNA adducts with respect to exposure to 0.16 µM pure B[a]P were observed for cells exposed to industrial and urban mixtures, respectively. These results show that components of environmental mixtures strongly impact the DNA damaging properties of PAH and that using only one genotoxicity test may be highly misleading. On the one hand, HPLC-MS/MS is so specific that it may neglect important classes of lesions. On the other hand, Comet assay is definitively not a global genotoxicity test like often presented although it is a convenient and easy technique.

Effect of Polycyclic Aromatic Hydrocarbons (PAHs) xenobiotics metabolism on DNA integrity, Development of a new genotoxic assay

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Several lines of evidence indicate that diet and dietary behaviours can contribute to human cancer risk. The aim of our study was to clarify the effect of food xenobiotic at low doses on DNA integrity. For this purpose we used three different human cell lines representative of the digestive tract: HepG2 (Liver), Caco2 (Colon) and ACHN (Kidney). We study the polycyclic aromatic hydrocarbons (PAHs) since they can be possible food contaminants. These xenobiotics comprise a large class of structurally related environmental chemicals that differ greatly in their carcinogenic potency. We used Benzo(a)pyrene (BaP), Fluoranthene (FLA) and 3-Methylcholanthrene (3-MC).

All these molecules were study for their metabolism in the three cell lines. We noted that HepG2 and Caco2 cell lines could metabolised all the PAHs tested in the same manner, but that the ACHN cell line was not competent for the metabolism of these compounds. Moreover, we noted that FLA was poorly metabolised compared with BaP or 3-MC.

Because all genotoxic assays (Micronucleus, COMET) are not enough sensible to detect very few DNA damage, we try to identified genotoxicity by the detection of the phosphorylation of the histone H2AX that was considered as good biomarker of DSBs in the cell. We tested the phosphorylation of H2AX by In Cell Western. We could detect DSBs with low dose (0.1µM) of genotoxic PAHs BaP and 3-MC in metabolic proficient cells (HepG2 and Caco2 cell lines), but not with the non mutagenic compound FA or in ACHN Cell line. In conclusion, only highly metabolised HAPs (BaP and 3-MC in HepG2 and Caco2 cell lines) could induce DNA damage. We therefore tested genotoxicity of seven other HAPs in the HepG2 cell line at 0.1, 1 and 10µM. Your new genotoxic assay is proficient to detected genotoxic xenobiotic in low dose range and is able to screen a lot of compounds alone or in combinations.

Fluorescent *in situ* hybridisation on comets: Possible application to DNA damage and repair

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The comet assay is a simple and sensitive method for measuring DNA damage and repair. Upon electrophoresis, the DNA of lysed, agarose-embedded cells extends towards the anode in a structure resembling a comet, the relative intensity of the tail reflecting the frequency of DNA breaks. We have used fluorescent *in situ* hybridisation (FISH) to investigate the structural organization of the DNA within comet preparations and to study specific DNA sequences within comets. Using large-insert genomic probes and human Cot-I DNA we found that, under neutral electrophoresis conditions, the probed sequence of DNA is seen as a linear array, consistent with extension from a fixed point on the nuclear core or matrix. After alkaline electrophoresis, the appearance of the fluorescent probes suggests that linear DNA has coalesced into a granular form. Another direction that we are taking in FISH-comet technology is detection of DNA sequences with 'padlock probes' (circularisable oligonucleotide probes). We have applied probes that hybridise to *Alu* repetitive elements and to mitochondrial DNA. During the sequence of stages in the comet assay, mitochondrial DNA progressively disperses into the surrounding agarose gel, showing no tendency to remain with nuclear DNA in the comets. In contrast, *Alu* probes remain associated with both tail and head DNA and can be used as an alternative way of staining comets, allowing us to visualise either single or both single and double stranded DNA in comets. To study specific gene sequences within comets, we have made a list of repair genes of interest to us and, as a starting point, have designed padlock probes to target three of these genes: the 8-oxoguanine-DNA glycosylase-1 (*OGG1*) gene, the xeroderma pigmentosum group D (*XPD*) gene, and the hypoxanthine-guanine phosphoribosyltransferase (*HPRT*) gene. Our initial experiments resulted in strong signals with no unspecific non-comet-bound background. These results look promising, suggesting further experiments with gene specific probes.

Probing lesion-induced DNA conformational changes by pulsed EPR

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Over the last decade, the combination of pulsed Electron Paramagnetic Resonance (EPR) with pulsed ELDOR sequence has become a powerful tool to measure distances between paramagnetic species up to 8 nm. p-ELDOR exhibits major advantages compared to other methods commonly used for this purpose since it can be used in a disordered state and can provide accurate and quantitative distance distribution shapes. For these reasons, this spectroscopy is now extensively used to study various biological systems including proteins, RNA and DNA.

We have used this method to study conformational changes in DNA induced by various lesions. Our strategy is to synthesise doubly spin-labelled oligonucleotides incorporating various chemical modifications. Conformational changes can be deduced from the distance change between undamaged and damaged systems (Figure 1).

We will present several significant results involving conformational changes in oligonucleotides. As a first example, we will show the analysis of a B/A transition induced by solvent. Then we will analyse the effects of various DNA modifications (nick, gap, bulge, oxo guanine, abasic site analogues…) in terms of importance of conformational change and of structural modification. Results obtained by p-ELDOR will be compared with those obtainable by other techniques (in particular NMR).

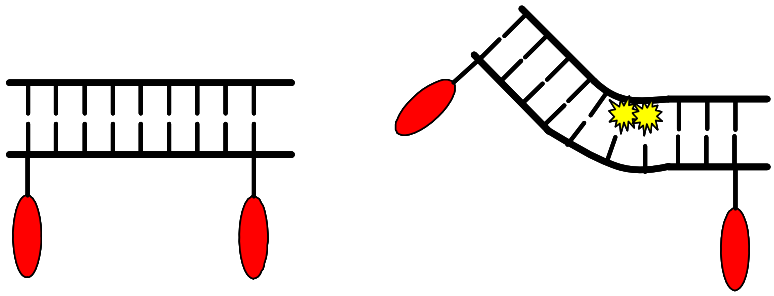

Figure 1: Strategy used to detect conformational change in damaged oligonucleotides

Chemistry and Biology of Complex Lesions Induced by Reactive Oxygen Species

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Reactive oxygen species can result in the formation of not only single-nucleobase lesions, but also complex lesions. Emphasis of this presentation will be placed on two types of complex lesions, tandem single-nucleobase lesions and intrastrand cross-link lesions where two adjacent nucleobases in the same DNA strand are covalently bonded. We will discuss the application of LC-MS/MS for identification and quantification of these complex lesions formed *in vitro* and in cells, and the use of shuttle vector technology for assessing the replication of these lesions in cells. We will reveal that some of the complex lesions can be induced in substantial levels and they can present significant challenges to the fidelity and accuracy of DNA replication.

Processing of clustered DNA damaged sites comprised of three lesions by the base excision repair pathway

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Ionising radiation (IR) induces clustered DNA damage, defined as two or more lesions within one or two helical turns of DNA, induced by a single radiation track. The base excision repair pathway is the main pathway by which non-DSB IR induced lesions are repaired, including clustered DNA lesions. Recent studies of clusters comprised of two lesions have established that the efficiency of repair of lesions within clustered damage sites is reduced. This present study was designed to study the repair of AP sites within clusters comprised of three lesions. Clustered damage sites, comprised of an AP site opposing a tandem cluster of two 8-oxoG lesions or a tandem 8-oxoG lesion and an AP site were synthesised in oligonucleotides and repair carried out in CHO nuclear extracts.

When a single AP site is opposite two tandem 8-oxoG lesions the repair of the AP site is retarded, the extent of the retardation being dependent on the relative position of the AP site and 8-oxoGs. If a second AP site is present on the opposite DNA strand in the 3' direction or greater than two bases in the 5' direction, in tandem with 8-oxoG, then there is rapid incision of both AP sites, resulting in the formation of DSB, regardless of the relative position of the 8-oxoG lesion. If, however, the second AP site is present two or less bases in the 5' direction on the opposing DNA strand, again in tandem with 8-oxoG, the incision of the AP site not in tandem with 8-oxoG is impaired. Interestingly the incision of the AP site in tandem with 8-oxoG is not influenced by the presence of 8-oxoG. This pattern of AP site incision leads to two populations of "repair" products, one that forms DSB and one that has a SSB in tandem with 8-oxoG and an AP site on the opposing DNA strand.

The clustered DNA damaged sites were engineered to be on a plasmid that was then transformed in to *E. coli*. The number of surviving *E. coli* colonies obtained from this plasmid based mutation assay reflects the biochemistry results. The clustered damaged sites that result in the formation of DSB do not support the survival of *E. coli* colonies. The number of surviving colonies obtained from the clustered damaged sites comprised of two tandem 8-oxoG lesions with an AP site on the opposing strand is comparable to the number of colonies formed from the control DNA. With these clusters, if the AP site strand was lost through replication induced DSB, following incision of the AP site, then the tandem 8-oxoG strand would still be able to support survival of *E. coli*.

These studies give insights into the biological effects of ionising radiation and how it can lead to mutations, and ultimately the formation of cancer. In addition, steps in the BER pathway may be identified that can be exploited for increased efficiency of the treatment of cancer by radiotherapy.

Base excision repair within nucleosomal substrates

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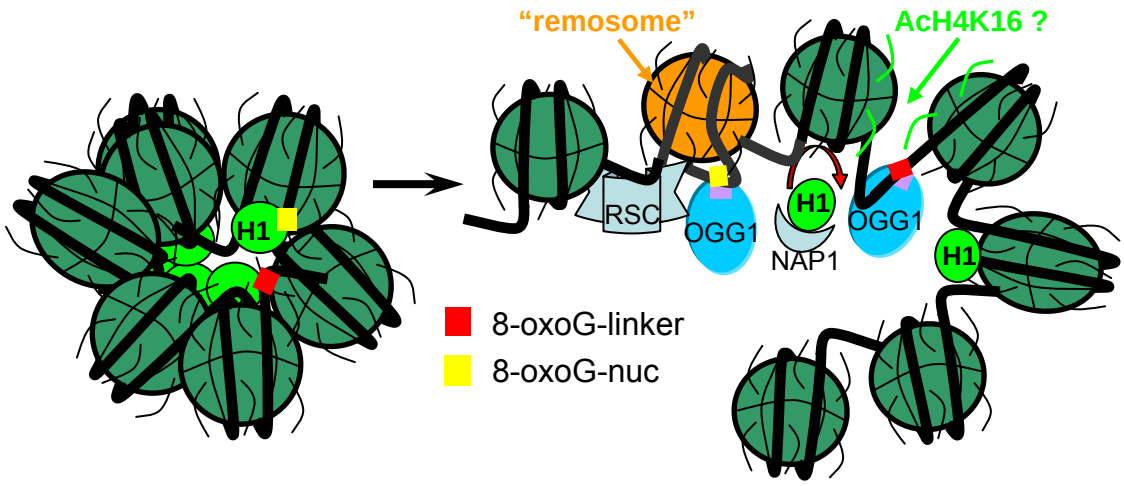
In eukaryotes, base excision repair (BER) is responsible for the repair of oxidative lesions. Here, we have studied the mechanism of BER by introducing a single 8-oxo-7,8-dihydroguanine (8-oxoG) in the DNA of a series of reconstituted positioned nucleosomal substrates ranging from mono-nucleosomes to compact 30 nm chromatin fibres. We found that both core histone as well as linker histone (H1) DNA interactions strongly inhibit BER of 8-oxoG located inside the nucleosome or in the linker DNA respectively. While the former inhibitory effect is overcome by the presence of ATP dependent nucleosome remodeling activity of SWI/SNF or RSC, the latter is alleviated by NAP1 specific eviction of H1. Interestingly, SWI/SNF induced "remodeling" without nucleosome mobilization was required to achieve the effect. These findings, together with our mechanistic study of the ATP-dependent nucleosome remodeling, lead us to proposal of a simple global-genome BER model, based on stochastic action of histone chaperones (NAP1) in conjunction with chromatin remodelers such as SWI/SNF and RSC.

Acknowledgements: Association pour la Recherche sur le Cancer (ARC), Région Rhône-Alpes (CIBLE).



DNA repair and free radicals: New insights into the mechanism of spore photoproduct lyase revealed by single mutation

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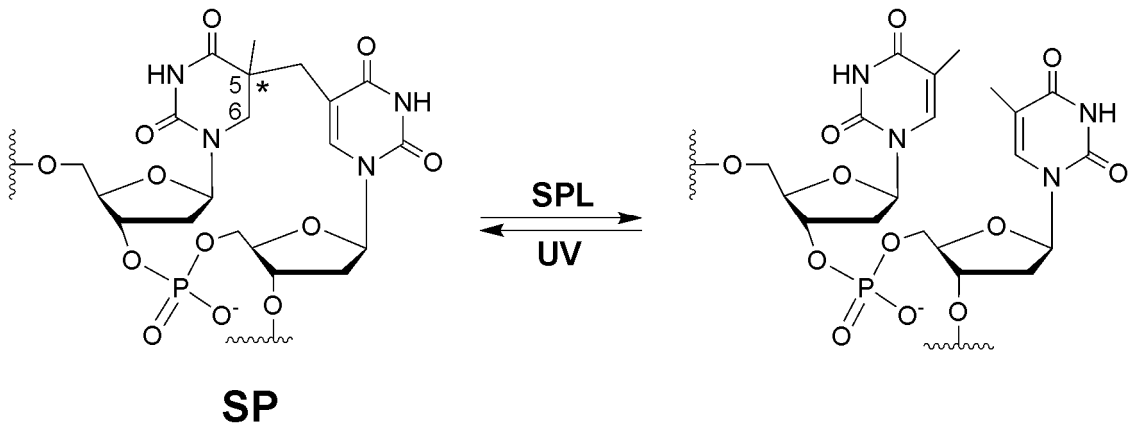
The DNA of all organisms is subject to modifications upon exposure to a wide variety of chemical and physical agents. Among them, solar ultraviolet radiation is known to induce dimerization reactions between adjacent pyrimidines. In spores of some bacteria such as *Bacillus subtilis* the only photoproduct generated upon exposure to UV light is 5-thyminyl-5,6-dihydrothymine (spore photoproduct, SP). The extreme resistance of spores to UV radiation is due to the presence of a specific and very efficient repair enzyme, the spore photoproduct lyase (SP Lyase) that directly reverts SP to two unmodified thymines upon germination (scheme). SP Lyase belongs to a superfamily of [4Fe-4S] iron-sulfur enzymes, named “Radical-SAM”, involved in a great variety of biosynthetic pathways and metabolic reactions that proceed via radical mechanisms. Recent biochemical and mechanistic studies by several groups and by our laboratory have provided detailed insights into the mechanism of the reaction catalyzed by SP Lyase and how this enzyme controls high potential intermediate free radicals. Recently we have shown that a single mutation at cysteine-141, a residue fully conserved in *Bacillus* species and previously shown to be essential for spore DNA repair *in vivo,* has a major impact on the outcome of the SPL-dependent repair reaction *in vitro.*



Scheme: UV-dependent formation and SPL-catalyzed repair of SP

Structure-function studies of DNA repair proteins from *Deinococcus radiodurans*

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Deinococcus radiodurans is a Gram-positive bacterium that displays an extraordinary resistance to ionising radiation and desiccation. *D. radiodurans* tolerates doses ranging from 5000 to 30,000 grays whereas most other organisms cannot survive doses above 50 grays. Such a massive radiation dose is estimated to induce several hundred double-strand breaks, thousands of single-strand gaps and about one thousand sites of DNA base damage per chromosome. Initial investigations support the view that the extreme radiation resistance of *D. radiodurans* is complex and most likely determined by a combination of factors such as efficient DNA repair machinery, genome packing and cell structure. To improve our understanding of this unusual phenotype, we are studying proteins involved in three major DNA repair pathways: base excision repair (BER), nucleotide-excision repair (NER) and the recombinational repair (RR) pathways. So far we have determined the three-dimensional structures of seven proteins in these pathways. Here we present the results of our studies of three BER enzymes (UNG, MUG, and AlkA), the recombinational repair protein complex RecOR and the nucleotide excision repair protein UvrA. Our structural data together with additional biochemical studies have improved our understanding of the function of these proteins in DNA repair and how they may contribute to the extraordinary radiation-resistance phenotype of *D. radiodurans*.

Monitoring small molecules impact on cell repair capability using lesion containing oligonucleotide microarrays

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Oligonucleotide microarrays containing DNA base lesions were developed to simultaneously evaluate the different glycosylase and/or endonuclease activities within a cell or tissue extract.

For the purpose of identifying glycosylase/endonuclease inhibitors, the existing test format was adapted to library screening automation and a positive control was prospected.

As preliminary experimentations, known base excision inhibitors and base analogue compounds were tested using HeLa nuclear extract.

Among compounds with high impact on excision activities (e.g. methoxyamine, nitro-indole carboxylic acid, aminouracil, carboxyuracil), hycanthone was ranked as the strongest one.

The test conditions were set to satisfy both excision efficiency (dependent on protein concentration) and inhibitory efficiency (dependent on hycanthone concentration).

Thus the best conditions were 6µg/ml of HeLa extracts for 250µM of hycanthone (the highest library compounds concentration possibly used).

In these conditions, the activities of the putative enzymes (NEIL1, UNG and/or SMUG, and APE1) responsible for the excision of thymine glycol, uracil (facing adenine and guanine) and tetrahydrofuran, were inhibited at 10%, 50% and 20% and 10% respectively.

A screening of 1120 molecules is actually running in the latter conditions (compounds at 250µM for 6µg/ml of protein).

We showed here that this multiplexed assay in its automated version seem very promising for the discovery of new cancer therapies and for screening in toxicology

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Repair and biological effects of spontaneous and ionizing radiation induced DNA damage

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Ionizing radiations (IR) induces a pleiotropy of DNA lesions including DNA-strand breaks of different complexity and oxidative damage to both sugar and base residues. There is good evidence that IR not only induces isolated single DNA lesions but in addition clustered DNA damage i.e. multiple single lesions formed within one helical turn in the DNA backbone. Although these clustered DNA damages might not induce at significant level by endogenous metabolic processes, the question is not yet definitely answered. In my presentation I will focus on both IR induced damage and spontaneous damage.

(i) We have investigated the induction, repair and biological consequences (genetic damage) of low (<100mGy) and high doses (>1Gy) of ionizing radiation, as well as the role of the Cockayne syndrome proteins in transcriptional bypass and repair of IR induced oxidative damage.

(ii) We assessed the impact of NER and translesion synthesis (TLS) on survival and mutagenesis in the mouse to gain information on the nature of spontaneous DNA damage in mammals.

The outcome of the study is that no threshold is found for genetic effects of low dose radiation, that IR has a differential effect on regulation of RNAPI (ribosomal) and RNAPII mediated transcription and that NER and TLS play a crucial role in suppressing spontaneous mutagenesis and toxicity in the mouse.

Transcription-coupled repair-deficient diseases: from cockayne syndrome to UV^{S}S syndrome

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Nucleotide excision repair (NER) is a versatile DNA repair system that removes a wide range of structurally unrelated lesions, including UV photoproducts, which result in large local distortions of the DNA helix. NER operates through two sub-pathways in the early stages of damage recognition, depending upon whether the damage is located anywhere throughout the genome (global genome repair, GG-NER) or in an actively-transcribed gene (transcription-coupled repair, TC-NER). The current model for TC-NER postulates that the pathway is initiated by the arrest of RNA polymerase II at a lesion on the transcribed strand of an active gene, in a process that requires several factors, including the CSA and CSB proteins.

Defects in NER are associated with three major autosomal recessive disorders, namely xeroderma pigmentosum (XP), Cockayne syndrome (CS), and trichothiodystrophy (TTD). At the clinical level, XP is characterized by a highly increased incidence of tumors in sun-exposed areas of the skin. In contrast, CS and TTD are cancer-free disorders characterized by developmental and neurological abnormalities, and premature ageing. The two genes identified so far as responsible for CS (*CSA* and *CSB*) are specifically involved in TC-NER. In addition, rare cases have been described showing a complex pathological phenotype with combined symptoms of XP and CS (XP/CS) that have been associated with mutations in the *XPB*, *XPD* or *XPG* genes.

NER defects have been reported in association with another disorder, designated UV-Sensitive Syndrome (UV^{S}S). The patients exhibit photosensitivity and mild skin abnormalities; their growth, mental development, and life span are normal and no skin cancers have been reported to date. At the cellular level, UV^{S}S and CS cells show similar responses to UV irradiation: increased sensitivity to the cytotoxic effects of UV light, reduced recovery of normal RNA synthesis rate (RRS) after UV irradiation and normal capability to perform UV-induced DNA repair synthesis. Two complementation groups have been identified in UV^{S}S patients, defined by mutations in an as-yet-unidentified gene in four cases and in the *CSB* gene in two individuals. In the latter cases, the mutation results in a severely truncated protein; however no CSB protein was detected by Western blot analysis, suggesting that the total absence of the CSB protein may be less deleterious than the truncated or abnormal counterparts found in CS-B patients. However, this hypothesis is not supported by recent investigations on two severely affected CS patients with undetectable levels of CSB protein and mRNA.

The recently identified French UV^{S}S patient (UV^{S}S1VI) with a mutation in the *CSA* gene may constitute a third complementation group of UV^{S}S. Furthermore, we showed that UV^{S}S1VI cells display normal cellular sensitivity to oxidative stress, in contrast to CS-A and CS-B fibroblasts. This finding supports the hypothesis that the striking differences between the pathological phenotypes of CS and UV^{S}S, namely neurological and physical deterioration with premature ageing features, are due to defective processing of oxidative DNA damage in CS but not in UV^{S}S patients.

Determination of the effects of aging and photoaging on DNA repair excision/synthesis capacities toward different DNA lesions using a functional biochip assay

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The skin as a physical barrier is directly in contact with exogenous sources of DNA damaging agents, notably ultra-violet radiations. Mechanisms underlying skin aging processes appeared highly complex due to cumulative effects of physiological aging and environmental-based assaults.

In this study we used our previously described functional biochip assay to measure excision/synthesis repair activities from fibroblasts from different donors. Emphasis was put on the effect of aging and of photo-aging on excision/synthesis features. For this purpose, biopsies taken from photo-protected (PP) and photo-exposed (PE) skin area of 33 volunteers were cultivated and nuclear extracts were prepared from the fibroblasts. Apart from age, all donors had similar characteristics (women, skin phototype 2 or 3, non smokers, moderate exposition to sun). Donors were classified in 3 groups of age (Group 1, 2, 3; mean age = 25; 46 and 65 respectively).

(1) Analysis of the repair assay by *group of age* revealed that there was a marked decreased of the repair with age whatever the DNA lesion considered (photoproducts, oxidized bases, alkylated bases, AP sites) and that the extend of the decline was dependent on the nature of the lesion.

Effect of photo-exposition was subtle. ANOVA revealed no statistical differences between the PP and PE cells whatever age group. However, statistical differences appeared for the repair of 8oxoguanine and AP site between PP and PE cells of Group 2 and 3 but only for certain amount of lesions (t test). Moreover, whereas in the middle age group repair of PE was superior to repair of PP, result was inversed in the older group.

(2) Analysis of *individual results* revealed that decline of repair was correlated with age for PP cells whatever the lesions considered (Spearman rank order correlation). On the contrary, no correlation was put in evidence in the PE group. Observation of the results showed higher dispersion of the data in the PE group compared to the PP group.

Hence our results showed that chronic aging is one of the major factor involved in the loss of repair capacity as level of excision/synthesis inversely correlated with age in the PP group.

However, this correlation was disturbed by sun exposure and the response was dependent on the individuals. These results underlined the heterogeneity of adaptive responses of individuals toward the UV genotoxic stress, with some individuals being able to respond by stimulating their repair response whereas for others, repair efficiency was affected.

Characterizing the parameters driving the heterogeneity of the repair response could be of the utmost importance to understand carcinogenic processes associated with sun exposure.

Base excision repair protein Ogg1 contributes to impaired transcription at the sites of oxidative base damage

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Oxidative DNA base lesions, particularly 7,8-hydroxy, 8-oxoguanine (8-oxoG), are intensely studied because of their mutagenic potential. Besides that, 8-oxoG has been recently shown to interfere with transcription machineries resulting in decreased gene expression and transcriptional mutagenesis. Our new data obtained from the transfections of the damaged plasmids suggest that roadblock for elongating RNA polymerases is likely to be absent at 8-oxoG sites under the physiological conditions of transcription in nucleus. We demonstrate instead that reduction of transcription occurs due to the processing of the lesions in cells and can be partially attributed to the incision of 8-oxoG by 8-oxoguanine DNA glycosylase (Ogg1) in the process of repair. Transcription of the damaged DNA is much more efficient in the presence of functional Csb protein, although not saved completely. The strategies that allow insertion of DNA lesions in the defined positions with respect to the transcribed gene are being exploited to understand the molecular mechanism for transcriptional inactivation of the damaged genes and the precise roles of DNA repair proteins in this process.

Role of an Alkyltransferase-like protein in *E. coli*: the *ybaZ* gene product enhances Nucleotide Excision Repair of O^6-alkylguanine adducts

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O^6-alkylguanine adducts are powerful pre-mutagenic lesions owing to efficient mutagenic bypass by replicative DNA polymerases that readily mis-insert thymine opposite these adducts. The strong mutagenic potential of these adducts is prevented by highly efficient repair pathways that remove O^6-alkylguanine adducts before replication converts them into mutations. A one-step repair pathway mediated by Alkyl transferases (AT) directly reverses these adducts by transfer of the alkyl group to a specific cysteine residue in the repair protein. E. coli possesses two such proteins (Ada and Ogt) that efficiently prevent O_6-methylguanine induced mutagenesis. In general, alkyl residues larger than methyl become poor substrates for reversion by AT and turn into substrates for the general nucleotide excision repair (NER) pathway instead. In this paper we have investigated the genotoxic potential in E. coli of the O_6-alkylguanine adducts formed by ethylene and propylene oxide using single-adducted plasmid probes. These adducts appear to be efficiently repaired owing to the overlap between AT and NER pathways as exemplified by the removal of O^6-hydroxyethylguanine adducts that is achieved by the action of both repair pathways. Our work demonstrates that a novel factor encoded by the ybaZ gene, a member of the Alkyltransferase Like (ATL) protein family, enhances repair by NER of some O^6-alkylguanine adducts via an interaction with UvrA. As ATL proteins bind to O_6-alkylguanine residues in DNA, YbaZ may act in a way similar to Mfd, the Transcription Repair Coupling Factor, by recruiting the $UvrA_2$.UvrB complex to the lesion site via its interaction with UvrA.

Error-prone repair pathways in immunoglobulin gene hypermutation

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Somatic hypermutation diversifies immunoglobulin genes during immune responses by a locus-specific mutagenic process. This process is initiated by the lymphoid-specific enzyme activation-induced cytidine deaminase (AID), which deaminates cytidines into uracils at the Ig locus. This initial lesion is further processed and gives rise to mutations at both G/C and A/T bases pairs, by recruiting translesional DNA polymerases along unusual repair pathways. Specific focus will be brought to the hierarchical recruitment of translesional DNA polymerases, with DNA polymerase eta constituting a dominant factor in error-prone repair processes generating mutations at A/T base pairs.

Oxidative lesions processing by high-fidelity DNA polymerases in *Pyrococcus abyssi*

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The hyperthermophilic anaerobe euryarchaeota *Pyrococcus abyssi* (*P. abyssi)* that thrives in extreme environments (elevated temperature, pH shifts, toxic metals and ionizing radiations) is supposed to be exposed to massive DNA damages and very high rates of potentially mutagenic DNA lesions (deamination, depurination, oxidation by hydrolytic mechanisms, alkylations and subsequent strand breakage) are expected to arise. However, and interestingly, it was demonstrated that the occurrence of abasic sites in the hyperthermophilic chromosome was moderately higher than the mesophilic bacteria, *Escherichia Coli*, posing the intriguing question of how this microorganism evolve to deal with a specific threshold of DNA damage (Palud *et al*, 2008). Among the limited number of DNA polymerising enzymes in *P. abyss*i identified to date, one eukaryotic-type primase and two replicative DNA polymerases families B and D have been described to functionally participate in the initiation and the elongation steps of DNA replication, respectively. Taken into account the absence of specialized translesional DNA polymerases and the persistence of DNA damage in the genome of *P. abyssi*, we speculate that one or both high-fidelity replicative DNA polymerases can be involved in DNA damage tolerance. In this study, we gave evidence of the occurrence of oxidative lesions in the genome of *P. abyssi* during normal growth or under stressful conditions. The processing of oxidative lesions is examined under running start and standing start reactions for the two *P. abyssi* DNA polymerases. In addition, preferential incorporation opposite oxidative lesions is employed to give preliminary insight on the mutagenic potential of such DNA lesions. These results are interpreted in terms of DNA damage tolerance.

Palud, A., Villani, G., L'Haridon, S., Querellou, J., Raffin, J. P., and Henneke, G. (2008) Intrinsic properties of the two replicative DNA polymerases of Pyrococcus abyssi in replicating abasic sites: possible role in DNA damage tolerance? *Mol Microbiol 70*, 746-61.

Repair of oxidized bases within multiply DNA damaged sites and mutation formation

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Multiply damaged sites (MDS) such as those generated by ionizing radiation comprises several (≥2) DNA lesions, including oxidized bases and strand breaks, distributed on both strands within ≤20 bp. It has been postulated that such clustered damage might hamper base excision repair (BER) process, the main pathway for the removal of radiation-induced base damage, or enhance double strand breaks (DSB) formed as repair intermediates and possibly leading to lethal events. To challenge these questions we synthesized MDS of increasing complexity; the most complex lesions are composed of 4 oxidized bases (including 8-oxoguanine, oG, and 5-hydroxyuracil, hU) and a 1 nt gap, distributed on both strands within 17 bp. Using mammalian whole cell extracts, we established that a hierarchy in the excision of base damage exists within MDS and depends on the nature and the distribution of the modified bases, as well as on the competition between the glycosylases involved in the excision of those modified bases [1,2]. The processing and the fate of MDS were analyzed in yeast *Saccharomyces cerevisiae*. Duplexes carrying MDS were inserted into replicative plasmid and used to transform yeast cells. Formation of DSB was assessed by a relevant transformation efficiency assay. None of the MDS carrying opposed oG and hU separated by 3 to 8 bp gave rise to DSB, despite the fact that some of them contained preexisting single-strand break (a 1 nt gap). Sequencing of the MDS-containing plasmid replicated in yeast showed an absence of large deletions, but a predominance of the lesion-targeted changes. We observed that the mutation frequencies at the oxidized bases within MDS were largely increased, as compared to single lesion. In conclusion, the inhibition of repair occurring at MDS is an important mechanism that prevents or limits the formation of deleterious repair intermediates like DSB. On the other hand, it dramatically enhances the probability to get point mutation, instead, a price to be paid for survival.

[1] Eot-Houllier et al 2005 Nucleic Acids Res. 33, 260-271.

[2] Eot-Houllier et al 2007 Nucleic Acids Res. 35, 3355-3366.

Genetic Instability induced by tritium contamination

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Because of its low disintegration energy, tritium biological effects cannot come from external exposure but from integration of organically bound (OBT) tritium into tissue. This results in an *in situ* chronic auto-irradiation. Consequently, radioactive compounds incorporated in tissues can have biological effects resulting from energy deposition in subcellular compartments. We addressed the genetic consequences of ^{3}H- or ^{14}C-thymidine incorporation into mammalian DNA on cell survival, DNA double-strand breaks (DSB), cell cycle, mutagenesis and homologous recombination (HR).

Low doses of ^{3}H-thymidine in CHO cells led to enhanced sensitivity compared with ^{14}C-thymidine. Compared with wild-type cells, homologous recombination (HR)-deficient cells were more sensitive to lower doses of ^{3}H-thymidine, but not to any dose of ^{14}C-thymidine. *XRCC4*-defective cells, however, were sensitive to both low and high doses of ^{3}H- and ^{14}C-thymidine, suggesting introduction of DNA double-strand breaks, which were confirmed by γ-H2AX foci formation. While γ-rays induced measurable HR only at toxic doses and high dose rate, sublethal contamination with ^{3}H- or ^{14}C-thymidine strongly induced HR. The stimulation level was in an inverse relationship to the relative contaminant energies. The RAD51 gene conversion pathway was involved as ^{3}H-thymidine induced RAD51 foci, and ^{3}H-thymidine-induced HR was abrogated by expression of dominant negative RAD51. In conclusion, both HR and non-homologous end-joining pathways were involved after labeled nucleotide incorporation (low doses) ; genetic effects were negatively correlated with the energy emitted, but positively correlated to the energy deposited in the nucleus, suggesting that low energy β-emitters, at non-toxic doses, may induce genomic instability. Moreover, we found oxidative stress induced by low doses of ^{3}H-thymidine responsible for mutagenesis induction.

Our results emphasize that the biological impact of tritium is conversely proportional to the isotope emission energy but correlate to the energy transferred to the nucleus. Taking together, the data presented here show that cell contamination with non-toxic doses of tritium may be hazardous for genetic stability. Thus, the remarkable survival of these contaminated cells associated to genetics alterations may increase the risk of: 1 - transmission of genetic modifications to the next generation and 2 - increase the risk of cancer (cancer cell should accumulated mutations and be viable to generate a tumour). Our work emphasizes the strong differences between an external ionizing radiation exposure and an internal radioactive contamination on biological consequences. The concept of dose of internal contamination in radiation protection may be re-evaluted.

Deinococcus deserti: Radiation tolerance and adaptation to harsh environmental conditions

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Deinococcus deserti is a radiation- and desiccation-tolerant bacterium isolated from surface sand of the Sahara desert, a nutrient-poor extreme environment exposed to harsh conditions such as UV radiation, temperature extremes and desiccation. To learn more about the adaptation to these conditions and about its extreme radiotolerance, we determined and analyzed D. deserti's entire genome sequence.

The remarkable tolerance of D. deserti to prolonged desiccation and high radiation doses is linked to efficient repair of massive DNA damage, as observed in D. radiodurans. However, the mechanisms underlying this repair and extreme resistance are not completely elucidated. The characterization of several Deinococcus-specific proteins involved in radiotolerance is in progress. More specifically, we found in D. deserti an important diversity of protein homologues. Compared to other Deinococci, D. deserti possesses supplementary genes involved in manganese and nutrient import and in DNA repair. Among the latter, two additional recA and three translesion DNA polymerase genes are most remarkable. Expression of the three recA and of two translesion polymerase genes was induced after exposure to UV, and their gene products were shown to be functional. These supplementary genes are likely important for survival and adaptation of D. deserti to its nutrient-poor and UV-exposed extreme environment.

The anticancer effect of selenium: study of its role in DNA repair activity and resistance to UVA

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Selenium is an essential trace element which is present in trace amount in foods (Brazil nuts, oysters, tuna fish…). Selenium acts as a cofactor for several antioxidant enzymes such as glutathione peroxidase and thioredoxin reductase, which have important antioxidant and detoxification functions. Several studies showed that increased selenium in the diet might be beneficial against liver, colon, pancreas and prostate cancer. Data reported from the Nutritional Prevention of Cancer (NPC) clinical trial indicated that dietary supplementation with 200 µg/day of selenium resulted in a 63% reduction in cancer risk. Mechanisms for selenium-anticancer action are not fully understood; selenium could oppose to toxic effects of free radicals on DNA which could be responsible for the genesis of certain malignant diseases.

Our main objective is to study the effect of selenium on DNA repair activity in order to understand the mechanisms of its preventive effects on cancer. Firstly, we found that pre-treatment of the human prostate cancer cells (LNCaP) with 30nM sodium selenite for 72 h protects them from UVA induced cell death. When selenium was added after irradiation, little protection could be achieved. Secondly, using the comet assay, we demonstrated that this pre-treatment decreased DNA damage. To distinguish between basal and oxidative DNA damage we used a modified comet assay with FPG enzyme. We showed that basal and oxidative DNA damage are decreased by 40% and 350% respectively in selenium treated cells.

These experiments demonstrate that a nano-quantity of selenium could protect cells against UVA toxicity. Additionally selenium decrease DNA damage, in particular oxidative DNA damage.

This project will allow us to a better understanding of mechanisms by which selenium could protect us against cancer.

Oxidative Stress, DNA damage and male infertility

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A growing number of data makes oxidative stress and related DNA damages a major cause of male infertility and failure for in vitro fecundation. An increase in DNA damage explains the relationship between ageing and decrease in fertility. In sperm DNA is partly protected by a very particular packaging due to the replacement of histones by protamines. These thiol reach proteins which are reticulated by disulfide bridges created by the selenoenzyme GPX4, strongly compact and imprison DNA. Oxidative stress occurs by intrusion of polymorphonuclear cells in sperm secondly to infection or inflammation, by exposure to toxic xenobiotics such as cadmium, by metabolic abnormalities such high glucose, by overstimulation of spermatozoa mitochondria, or by a decrease of the antioxidant capacity of spermatozoa or seminal fluid (deficiency in zinc, selenium or tocopherol). Spermatozoid is protected by various glutathione peroxidases and superoxide dismutases. Seminal fluid is rich in small thiol compounds, GPx5 and vitamin C. An abnormal level of DNA damage is found in one third of men after 35 years old, as measured by comet assay or by terminal transferase (to detect strand breaks) or by measuring 8oxodG. Consequences of DNA damage are a decrease in mobility or number of spermatozoa, morphological abnormalities of spermatozoa (all decreasing fertility), but also can have more dramatic results for children such as embryo rejection, abnormal development of the embryo and malformations, or latter obesity or cancer. Fortunately most of the DNA damages will be repaired by female DNA repair systems, after penetration inside the oocyte. But modern techniques for procreation such as Intracytoplasmic sperm injection (ICSI) increase the danger by directly injecting in the oocyte a strongly damaged male DNA from an abnormal spermatozoa who naturally will never have penetrate the oocyte membrane. So it seems logical to treat infertile people with antioxidants to prevent DNA damage. But we have to be careful when supplementing, as H2O2 is necessary to permit GPX4 to oxidize the thiols of protamin, compacting DNA. An excess of antioxidant supplementation has been described as decondensing DNA and paradoxically decreasing fertility. Monitoring DNA damage and DNA decondensation of spermatozoa is now necessary when exploring or treating male infertility.

Oxidative DNA Damage in Barrett's Oesophagus

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Barrett's oesophagus (BE) is the recognised precursor for oesophageal adenocarcinoma (EA) and is strongly associated with exposure to reflux. Methylene blue chromoendoscopy is used to aid the targeting of biopsies in Barrett's tissue for diagnostic purposes. This technique results in increased levels of DNA strand breaks and Fpg sensitive sites in Barrett's tissue as detected by the alkaline comet assay. A simple modification to current endoscopes may limit DNA damage during this technique and will be described.

There is increasing evidence to suggest that the development of BE and EA is associated with free radical production and poor antioxidant status. We have shown that nitric oxide production occurs in an oesophageal cell line exposed to the bile acid, deoxycholate. This results in DNA strand break formation but this can be inhibited by the addition of the nitric oxide synthase inhibitor, L-NMMA or by sequestering NO radicals.

Analysis of Barrett's oesophagus tissue reveals increased levels of DNA strand breaks and Fpg-sensitive sites and reduced vitamin C levels compared to matched normal squamous oesophageal tissue. Vitamin C supplementation in Barrett's oesophagus patients for 3 months did not reduce levels of DNA strand breaks or Fpg sites.

Possible link between oxidative DNA damage and cancer: i/ case of *BRCA1* mutation carriers, ii/ relationship between DNA oxidation and methylation

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Although there is a little room for doubt on the basis of available experiments that oxidative DNA damage has some role to play in the pathogenesis of cancer, the direct relationship between the measured DNA damage and the rate of mutation and cancer is still lacking. In our presentation some evidences which suggest that oxidative DNA damage may be causal factor in carcinogenesis will be presented.

i/ Carriers of *BRCA1* mutations are predisposed to develop a lifetime risk of breast cancer of about 80% and ovarian cancer of about 40%.Therefore, to asses an involvement of *BRCA1* in oxidative damage to DNA and to have a further insight into the issue concerning a role of the damage in cancer development, we measured the parameters reflecting oxidative DNA damage in three groups of subjects: a/ the group of healthy subjects; b/ patients with *BRCA1* mutation without symptoms of the disease and; c/ patients with breast or ovarian cancer with the *BRCA1* mutations.

The results of our study demonstrated that *BRCA1* mutation carriers have elevated level of promutagenic 8-oxo-2'-deoxyguanosine (8-oxodG) in DNA of lymphocytes, what in turn suggest that the damage may be a factor responsible for cancer development.

ii/ It has been known for a long time that global DNA hypomethylation occurs in many human cancers and precancerous conditions. This has led to the suggestion that the hypomethylation might be responsible for enhanced genetic instability and cancer development. However, the mechanisms of hypomethylation are largely unknown. It is also unclear whether this epigenetic phenomenon is a cause or consequence of cancer development. It is also possible that 8-oxodG level may be linked to aberrant DNA methylation and in this way influence carcinogenesis. The aim of our study was to assess a possible link between the levels 8-oxodG and 5-methyl-2'-deoxycytidine in DNA. This relationship was determined for the first time *in vivo,* in DNA from human leukocytes of healthy subjects as well as in patients with colon adenomas and carcinomas. Our results support earlier reports which demonstrated that global DNA demethylation is associated with cancer development and extend these observations showing that oxidative DNA damage, namely guanine oxidation, may be a factor influencing demethylation process *in vivo,* which may predispose to disease development.

Repair of oxidative DNA damage and the risk of tobacco-related cancers

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Mutations are critical for the development of cancer, and therefore processes that guard cells against excessive mutations, like DNA repair, are expected to be important in protection against cancer. This is indeed the case in several human hereditary cancer predisposition diseases, such as xeroderma pigmentosum, where the predisposing mutations were found in DNA repair genes. In sporadic cancers, which account for the majority of human cancer cases, DNA repair is expected to play an important role too, however its elucidation is much more complex. We have developed an epidemiology-grade assay for the enzymatic activity of the DNA repair enzyme 8-oxoguanine DNA glycosylase (the OGG assay), and we are currently finalizing the development of three additional DNA repair tests. We have previously shown that reduced OGG enzymatic activity is associated with the risk of lung cancer (Paz-Elizur *et al* 2003, *J. Natl. Cancer Inst.* 95, 1312-1319) and head & neck cancer (Paz-Elizur *et al* 2006, *Cancer Res.,* 66, 11683-11689). Preliminary analysis of the origins of the inter-individual variation showed a poor correlation between enzymatic activity and mRNA expression of OGG1 (Paz-Elizur et al 2007, *DNA repair*, 6, 45-60). For both cancers the combination of smoking and low OGG further increased the risk of the disease. Such studies pave the way to use DNA repair diagnostics for risk assessment and early detection of lung cancer.

Abstracts

Posters

Optical excitation and detection of singlet oxygen: Towards intracellular microscopy of reactive oxygen species

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The objective of this project is the study of the spatially resolved cellular responses of singlet oxygen in biological systems. In the Photodynamic therapy, molecular oxygen in its singlet excited state acts like a radical and destroys interactions between functionnal molecules in living cells. Monitoring its creation inside the cell would allow to study the dynamics of oxydative stress leading to cell death. Our studies are based on the development of a new time-resolved microscopy in the infrared to detect the 1270 nm singlet oxygen fluorescence.

As a first step to this project, strategies to produce optically (and detect temporally) singlet oxygen without photosensitiser will be presented. Preliminary results on oxidative and thermal stresses on female breast cancer cells (MCF - 7) death upon laser irradiation will be presented and discussed.

Phytochemicals affecting DNA repair

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Dietary antioxidants may be in part responsible for the cancer-preventive effects of fruits and vegetables but their role remains controversial. These effects are often attributed to their content of antioxidants, and a resulting protection against oxidative stress, but human intervention trials have failed to confirm their protective effects and mechanistic studies have given inconsistent results. In this study we have investigated the effect of physiological concentrations of vitamin C and β-cryptoxanthin on DNA repair of HeLa cells using two approaches based on the comet assay. In the first one cells are subjected to damage, incubated to allow the repair and the comet assay is performed at intervals to measure the residual lesions (cellular repair assay). In the second one a cell extract is incubated with agarose-embedded substrate nucleoids containing specific damage. The ability of the extract to induce breaks in the substrate nucleoids indicates the activity of the enzymes responsible for the initial steps of repair - incision (in vitro repair assay). Vitamin C has no effect on cellular repair of strand breaks (SBs) or oxidised purines while β-cryptoxanthin leads to a doubling of the rate of rejoining of SBs, and has a similar effect on the rate of removal of oxidised purines. The in vitro assay could not be carried out with vitamin C since it was evident that vitamin C itself can cause damage to nucleoid DNA. Cells incubated with β-cryptoxanthin have a higher incision activity than the ones non-incubated. This stimulation on DNA repair may help to explain the cancer-preventive effects of some carotenoids.

The increase of oxidative stress after transient glutathione depletion and irradiation triggers apoptosis in head and neck squamous cell carcinoma

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Glutathione, a major intra-cellular antioxidant, has been reported to play a fundamental role in the resistance of some cancer cells to radiotherapy so that this molecule can be considered as a potential clinical target for their radio-sensitization.

The radio-resistant head and neck squamous carcinomas cell line SQ20B was shown to display a high endogenous level of reduced glutathione, up to 8-fold when compared to its radiosensitive counterpart SCC61 cell line. This result led us to consider the endogenous reduced glutathione as a key factor involved in the resistance of SQ20B cells to ionizing radiation. The purpose of this study was to experiment the incidence of increasing the oxidative stress in SQ20B cells by a short and transient depletion of intracellular glutathione before irradiation. We therefore made use of the combination of dimethylfumarate (DMF), a glutathione-depleting agent in association with buthionine sulfoximine (BSO), a specific inhibitor of glutamate-cysteine ligase, the first enzyme of the glutathione biosynthesis pathway. In preliminary experiments, we have determined both the efficiency in lowering the endogenous glutathione level and the potential cyto-toxicity of these molecules in un-irradiated SQ20B cells. Although no toxicity was evidenced, a 95 % decrease of intracellular glutathione was obtained under our experimental conditions.

This treatment combined to irradiation induced an increase of oxidative stress through a higher rate of DNA damage radiation-induced and a partial inhibition of repair mechanisms. Moreover, this combination led to the triggering of apoptosis of the radioresistant SQ20B cell line which significantly increased from 48 hours to longer times, as evidenced by an enhancement of 50 % of the activity of caspases and of the number of cells in the sub-G1 phase. Triggering of apoptosis in SQ20B cells was found to involve the *c-Jun N-terminal Kinase* (JNK) pathway which was phosphorylated 1 h after irradiation. This activation of JNK was dependent upon the generation of radical oxygen species which resulted in the dissociation of thioredoxin-ASK 1 complex (MAPKKK of JNK pathway). The phosphorylated form of JNK led to the activation and translocation of Bax to mitochondria which further resulted in the alteration of the organelle, as evidenced by the loss of the mitochondrial transmembrane potential and the increase of the secondary radical species.

Taken altogether, our results demonstrate that a transient glutathione depletion before irradiation can trigger apoptosis in radio-resistant SQ20B cells through the activation of a JNK-dependent pathway. Moreover, the absence of a significant toxicity of this pharmacological treatment in tumours cells and normal keratinocytes, is a fundamental importance for potential clinical application.

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Practicability of the Comet assay to investigate the influence of flavonoids on DNA repair kinetics

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Equilibrium between DNA damage and repair appears vital to the cellular machinery, to health and longevity. Given the complexity of DNA repair and the high number of proteins and cofactors involved, it seems possible that xenobiotics, from food, spices, medicinal herbs or drugs, may interfere in the process, activating or inhibiting repair.

To determine if the highly alkaline comet assay can be used for the screening of DNA repair modulation by natural products, flavonoids from 5 different structural classes were investigated. Indeed, flavonoids constitute a highly investigated group of secondary metabolites particularly abundant in our diet and many of these compounds present a number of biological properties, including antioxidant and cancer prevention activities.

Two types of experiments were performed using the highly alkaline comet assay : investigation of direct repair kinetics (ethyl methanesulfonate [EMS] damage of cells, followed by repair incubation in the presence of flavonoids) and *ex vivo* experiments (measurement of excision capabilities of protein extracts obtained from flavonoid-treated cells on EMS-damaged nucleoids).

Some flavonoids appear able to modulate the repair of DNA alkylated damage. A consistent effect is shown by the flavone apigenin that induces endonucleases able to excise this kind of damage, which may lead to an apparent faster repair; this is indicated by lower strand breakages at all investigated time points (12h, 24h, 48h). The flavonol quercetin induces non-specific endonucleases, resulting in a higher strand breakage at a short time; this is consistent with a clastogenic effect of this flavonoid. Direct repair and *ex vivo* experiments yield complementary data that may lead to mechanisms characterizing. However, labor-intensive manipulations make the method less suited for screening. These data suggest that some major dietary compounds may promote DNA repair, which could help to prevent the carcinogen risk.

Pulsed EPR structural analyse of DNA damages caused by platinum anticancer agents

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DNA is known to be a favoured target for platinum anticancer agents, such as cisplatin and oxaliplatin (1). Such adducts lead to structural changes of the double stranded DNA molecule.

Traditional techniques used to study platinated-DNA structure are NMR, X-ray diffraction or FRET (Fluorescence Resonance Energy Transfer).

Here we present a new pulsed-EPR technique (Electron Paramagnetic Resonance) which was recently developed and applied to structural studies of DNA fragments (2).

Pulsed-EPR is based on the interactions between two paramagnetic centres and permits distances measurements as far as 80Å, within a precision close to one angstrom (3).

We have constructed paramagnetic double stranded DNA probes containing one platinated cross linked damage and two nitroxide groups. These groups were introduced by using a click chemistry approach. Structural deformations on DNA duplexes, caused by platinated derivatives, lead to a distance modification between the two paramagnetic centres in comparison with non-damaged duplex.

By combining the distances measurements with 3D-modelisation data the structural deformations induced by several platinated derivatives were determined.

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Synthesis and incorporation of carbocyclic analogues of the oxidative DNA-Lesion FaPyG

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Numerous endogenous and exogenous factors constantly damage the DNA in our cells. Oxidative stress in human cells generates a huge number of highly mutagenic lesions responsible for accelerated aging and the development of cancer.[1] Guanine has the lowest oxidation potential of the canonical bases and is therefore the preferred target for an oxidative attack.[2] Most of the oxidatively generated DNA lesions are repaired *via* the Base Excision Repair pathways (**BER**).[3] The nucleophilic attack e.g. of the N-terminus of a DNA-glycosylase like MutM/FPG cleaves off the damaged nucleobase. However the process of lesion recognition is not fully understood. Replacing the furane ring of the sugar by a cyclopentane ring leads to enzymatically uncleavable lesions.[4] Incorporation of these lesions into DNA and crystallization experiments together with enzymes give snapshots of the cleavage mechanism at the atomic scale. In order to study the biological relevance of DNA lesions, their basepairing and the damage recognition, both isomers of the carbocyclic lesion were synthesized and incorporated into DNA at a defined location and in an unequivocal conformation.[5,6]

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Design of a ligand-fishing method for the characterization of proteins associated to DNA lesions generated by cisplatin

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Many anti-cancerous treatments directly alter tumour cell DNA by forming covalent addition products with its bases, as well as DNA-DNA and DNA-protein crosslinks. Questions still need to be addressed regarding which proteins are involved in the recognition, repair or bypass of these chemical lesions. The goal of our study is to establish a molecular cartography of proteins involved in these processes for a specific damage. DNA lesions caused by *cis*-diamminedichloroplatine (II) (cisplatin) are well-characterized, with a majority composed of intrastrand GpG crosslinks. In addition, several proteins such as nucleotide excision repair components (XPC, XPA, ERCC1 ...) or high-mobility-group proteins (HMGB1, hUBF …) are already known for their capability to get directly or indirectly bound to cisplatin adducts. We therefore chose cisplatin as our model of damaging agent for optimization steps.

We have adapted a simple and straightforward ligand-fishing method to capture DNA-binding proteins [1]. It consists of linearized plasmid DNA linked to magnetic beads by a streptavidin-biotin interaction. Following exposition of this system to a DNA-damaging agent, it will serve as a trap for proteins contained in cellular lysates and which are associated with chemical lesions. HeLa nuclear extracts are the first biological lysate tested. A subsequent proteomics analysis on the recovered proteins will allow their identification.

The poster is presenting preliminary results regarding the following optimizations:

- molecular characterization of the ligand-fishing system (quantity of immobilized DNA, DNA adduct levels, ...)

- evaluation of several recovering methods to take off proteins from the fishing system

- evaluation of non specific binding both on beads and on target DNA

- trapping of proteins already known to be associated with cisplatin damage (HMGB1)

- identification of trapped proteins using proteomic tools.

Following validation on well-known DNA lesions and cellular models, we intend to apply our method to original DNA chemical lesions discovered and characterized in our laboratory as well as to other cellular models.

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Identification of a new (6-4) photolyase

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The *Cryptochrome-like protein 1* from *Ostreococcus tauri* was identified as a (6-4) photolyase. Reconstitution experiments with the photoantenna F0 showed a more efficient (6-4) photoreactivation.

INTRODUCTION

Ultraviolet light is a potent DNA damaging agent and generates highly cytotoxic photolesions that are associated with the incidence of skin cancer [1]. The most important types of photolesions induced by UV-B and UV-C light are the *cis-syn* cyclobutane pyrimidine dimers **1** (CPD photoproducts) and pyrimidine(6-4)pyrimidine ((6-4) photoproducts) photolesions **2** and **3**. These two types of photolesions are responsible for 70-80 % and 20-30 % of the total amount of UV induced lesions, respectively [2-4]. Other generated photoproducts include the Dewar valence isomers **4** and **5**, which are formed by further irradiation of the (6-4) photoproducts **2** and **3** with UV-A/B light [5,6].

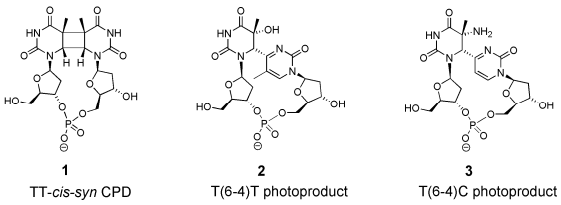
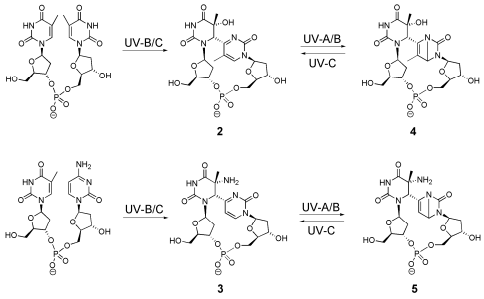


Figure 1: Main DNA photoproducts induced by UV-B/C irradiation.



Scheme 1 : Formation of (6-4) photoproducts **2** and **3** and its Dewar valence isomers **4** and **5** under UV light.

RESULTS AND DISCUSSION

Ostreococcus tauri is the smallest free-living eukaryote belonging to the most ancient group of organisms within the evolutionary lineage of green plants [7, 8]. In the present work the expression and purification of a recombinant *Cryptochrome-like protein 1* from *Ostreococcus tauri* is described. Coirradiation with a 8mer *ss*DNA containing the T(6-4)T photoproduct revealed its (6-4) photoreactivation activity. The protein was obtained without a second light harvesting cofactor (F_0). Determination of the repair activity before and after reconstitution with F_0 revealed that binding of the cofactor increases the repair efficiency by a factor of 5.

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Oxidative DNA damage induced by benzo(a)pyrene in the testis of Ogg1-deficient mice, and repair of such lesions *in vivo*

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The importance of the paternal genome for fertility and health of the offspring has become increasingly evident during recent years, and negative conditions related to the reproductive organs are increasing in the industrialised world. Benzo(a)pyrene (BaP), a carcinogen present in i.e. cigarette smoke, diesel exhaust and smoked food, is one agent proposed to have negative effects on reproduction. BaP is known to mediate bulky DNA adducts, but is also believed to induce oxidised base damage indirectly through generation of reactive oxygen species (ROS). We have shown that human male germ cells exhibit limited repair of oxidised DNA such as 7,8-dihydro-8-oxodeoxyguanosine (8-oxoG), lesions mainly repaired in other cell types via base excision repair (BER). A central enzyme for this removal is 8-oxoguanine-DNA glycosylase (Ogg1). We use mice deficient in this gene to mimic the normal human male with respect to repair of oxidative DNA lesions in the testis. In this study we have measured repair *in vivo* of oxidised DNA lesions (Fpg-sensitive DNA lesions) in *Ogg1*$^{-/-}$ and *Ogg1*$^{+/+}$ mice exposed to X-rays (10 Gy) using the comet assay. The results indicate no significant back-up repair by alternative repair pathways. We have examined whether exposure to BaP (150 mg/kg i.p.) leads to the induction of oxidative DNA lesions in the testes *in vivo,* in both *Ogg1*$^{-/-}$ and *Ogg1*$^{+/+}$ mice. Furthermore, we observed various changes in the gene expression patterns of *Akr1a4*, *Cyb1a1*, *-1a2* and *-1b1,* involved in the metabolism of BaP using real-time RT-PCR. We found that BaP did induce increased levels of oxidative DNA damage that were statistically significant in the testis of *Ogg1*$^{-/-}$ mice. Our results indicate that, unlike rodents, human male germ cells are particularly susceptible to oxidations that will not be repaired. This could explain the elevated levels of such lesions observed in sperm of smoking men. We speculate that environmental agents may induce DNA lesions in human male germ cells that confer negative effects on fecundity, fertility and possibly also the health of the coming generations.

Damaged-oligonucleotides biochip: a convenient means to assess the DNA repair ability of any biological sample

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Various DNA repair mechanisms, including the Base Excision Repair (BER) pathway, act in cells to maintain genome stability. Defects in these DNA repair processes often lead to carcinogenesis. As many drugs commonly used for anti-cancer treatments do their work by damaging DNA, DNA repair may also have a critical role in response to chemotherapy. Profiling the repair capability of cancer cells is thus a crucial issue for cancer susceptibility diagnosis. Therapeutics is also concerned as a better knowledge of BER in response to chemotherapy (inhibition or stimulation) could allow adjusting treatment to maximize effectiveness while minimizing toxicity.

To this aim, we developed a multiplexed assay giving access to the repair profile of any biological sample. This biochip, functionalized with 13 different lesion containing oligonucleotides, allows a simultaneous evaluation of excision capacity of most of enzymes involved in BER (UNG, APE1, AAG, hNTH1, OGG1, MYH…).

Our work currently focuses on the characterization of various cancer cell lines (RPMI 8226, HCC 1937, HCT 116…). Our ambition is first of all to characterize their DNA repair profile in correlation with their genotype, but also to evaluate the incidence of standards of care agents used in chemotherapy on BER pathway.

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DNA Cleavage by ditopic copper-platinum complexes

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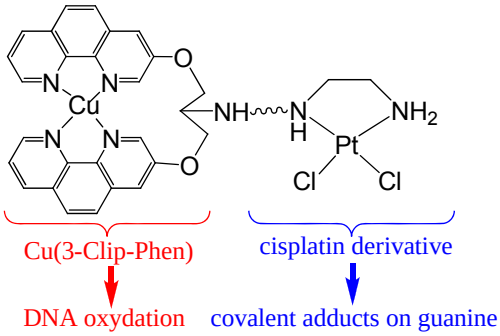
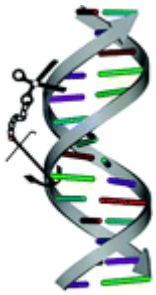
Cisplatin and bleomycin are efficient anticancer drugs that target DNA but their mechanism of action is different. Cisplatin primarily induces distortions upon binding to DNA whereas Fe(bleomycin) generates DNA strand scissions. Since the discovery of bleomycin, numerous metal complexes, such as Cu(3-Clip-Phen), have been synthesized that are able to produce single-strand DNA cleavage by oxidation of the deoxyribose units.

We have synthesized new bifunctional heterodinuclear platinum-copper complex. The design of this ditopic coordination compound is based on the specific mode of action of each component, namely cisplatin and Cu(3-Clip-Phen). The sequence selectivity has been investigated by primer extension experiments which revealed that its interaction with DNA occurs at the same sites as cisplatin. Cisplatin is not only able to direct the Cu(3-Clip-Phen) part to the GG or AG site, but also acts as a kinetically inert DNA anchor. The nuclease activity study showed that the dinuclear compound is not only more active than Cu(3-Clip-Phen), but is also capable to induce direct double-strand breaks. Sequence selective cleavages have been also studied. They were observed in the close proximity of the platinum sites.

We present here primary results concerning the analysis of these DNA modifications at a molecular level.





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Protein complexes assembled in vitro at double strand breaks

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We recently elaborated a straightforward method for single-step hyper-specific DNA-based purification of nucleic acid-protein complexes[a]. The duplex oligonucleotide bait mimics a double-strand break at one end and recruits putative repair complexes when incubated with nuclear extracts. The other end of the duplex is functionalized with a biotin via a photo-cleavable linker so as to ensure strong interaction with streptavidin-coated magnetic beads. Once purifed, the native nucleo-protein complexes are decoupled from the beads upon UV-irradiation. The proteins therein were identified by MALDI-TOF /TOF mass spectrometry after direct tryptic digestion of purified nucleo-protein complexes or in gel tryptic digestion of spots from blue native-SDS-PAGE gels. The chemical nature of DNA ends (directly ligatable or with complex chemistry mimicking radiation damage) for the recruitment of specific proteins is under investigation. The complexes assembled on DNA ends have been compared using nuclear extracts from basal or activated cells submitted to a radiomimetic compound (orthophenanthrolin) inducing genomic strand-breaks by radicalar mechanisms.

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Characterization of DNA repair phenotypes from XPA and XPC cell lines with an *in vitro* miniaturized test

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Xeroderma pigmentosum is one of the most deleterious DNA repair diseases where the skin of patients is highly sensitive to UV irradiation. XP patients are unable to efficiently take in charge several DNA lesions including bulky and small base lesions. In order to simultaneously characterize Nucleotide Excision Repair (NER) and Base Excision Repair (BER) efficiency from cell lysates, we have developed a highly sensitive microarray plasmid chip assay aimed at quantifying excision/synthesis (ES) activities. Quantitative measurements of cell repair capacities toward several base lesions (photoproducts, cisplatin adducts, oxidized bases and abasic sites) are obtained simultaneously. ES phenotypes of nuclear extracts from XPA and XPC fibroblasts were established using this assay. As expected, XPC nuclear extracts showed marked impaired repair capacities whatever the lesion considered and the experimental conditions applied. In particular, ES efficiency was directly protein-content independent. Contrary to what is generally admitted, XPA exhibited residual ES activities toward all the lesions tested. Strikingly, repair of 8oxo-guanine was more efficient for XPA extract than for the control cell line extract. However, the XPA ES efficiency, with respect to the control cell line extract, was highly dependent on the protein concentration assayed. More specifically, impaired repair of Cisplatin adducts and Photoproducts was observed, but only using high protein content. These results are in favour of a complex regulatory role for XPA during DNA repair that could act as negative regulator for certain steps.

The role of XPC and XPA proteins and the influence of protein concentration in *in vitro* DNA repair assays will be discussed.

We are grateful to Pr. Alain Sarasin for kindly providing us the XP cell lines and to the transversal CEA programme “Technologie pour la Santé” for its financial support.

Response of cancer cell lines to genotoxic drugs in relation to chemoresistance : DNA repair phenotyping using a functional multiplex miniaturized assay

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The acquisition of drug-resistance in tumour cells is a major problem in the medical treatment of a range of malignant diseases. To assess the role of DNA repair in the acquired or innate resistance, we characterized the basal level as well as the response of 8 different tumour cell lines (colon carcinoma HCT-116 and HCT-15, bladder carcinoma T24, breast carcinoma HCC-1937, MCF-7 and MCF-7/ADR, myeloma RPMI 8226 and RPMMI/Dox) toward a panel of five anti-cancer genotoxic drugs (cisplatin, oxaliplatin, adriamycin, 5-fluoro-uracile and carmustine). For this purpose, we used an innovative multiplex functional assay that allowed the phenotyping of DNA repair excision/synthesis capacities of tumour cell extracts, using a fluorescent assay on biochip. Treatment was performed over a 72h period, at IC20 concentration at 72h, for each drug. Two normalized parameters were chosen to characterize the repair phenotype: the global repair capacity expressed as a total fluorescent level, and the contribution, in percentage of the total repair, of the repair of each of the 6 categories of DNA lesions present on the biochip (cisplatin-adducts, UV-induced lesions, oxidized bases, abasic sites,…). We found that at basal level each cell line exhibited a typical DNA repair profile. Importantly, low basal level of repair did not preclude efficient response to drugs. Effect of the drugs on DNA repair (stimulation, inhibition) was not necessarily correlated with the corresponding cytotoxicity. Our results also indicated that each drug induced a modulation of the repair phenotype dependent on the nature of the drug and on the characteristics of the tumour cells. Roughly, two categories of responses were observed: (1) global effect of the drugs on repair capacity with no modification of relative efficiency of the different repair pathways, (2) specific drug effect on certain repair pathways. Repair phenotypes were compared to available data on mechanisms responsible for known cell resistance, on known mutations in cell regulatory and repair genes. In conclusion, repair phenotype together with the knowledge of mutations in key genes could help predict the sensitivity of cell lines to specific drugs. This new approach of DNA repair phenotyping could be a major breakthrough for the understanding of the complex response of tumours to chemotherapy. Moreover, it could be the first step toward the use of DNA repair phenotypes as a predictive marker of individual responses to chemotherapy and could encourage the emergence of new strategies to overcome drug resistance.

This work was supported by the CEA transversal program “Technologies pour la Santé”.

Rad14 confers specificity for bulky adducts in Nucleotide Excision repair

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DNA is the universal carrier of genetic information in all kingdoms of life. The preservation of its integrity is essential to the survival of any species. To sustain this integrity, a network of multiple DNA repair pathways has evolved. One of the most versatile and sophisticated DNA damage removal mechanism is nucleotide excision repair (NER), which can remove a wide range of lesions. Despite the fact that all key factors of NER have been identified in yeast, the mechanism of damage recognition is still not fully understood. Rad4/Rad23 and Rad14 are known to be responsible for the damage recognition in *Saccharomyces cerevisiae*, however the damage-specificity of these proteins has not been thoroughly investigated, yet. In this study, we examine binding of purified Rad14 to various defined DNA lesions, such as aminofluorene and cisplatin adducts as well as UV photoproducts and the widely used fluorophore fluorescein. Our results show that Rad14 discriminates between damaged and undamaged DNA, as well as different lesions, respectively. We demonstrate that Rad14 specifically recognizes bulky adducts, preferably hydrophobic aromatic systems like aminofluorene and fluorescein and to a minor extend cisplatin adducts. Based on these results, we suggest that Rad14 plays an important role in the primary identification of this type of lesion. Whether there are other specificity conferring factors for other lesions remains to be elucidated.

Base excision repair-induced single strand breaks initiate Rec12-independent meitoic recombination

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Thymidine-DNA glycosylase (TDG) and uracil-DNA glycosylase (UNG) excise uracil from DNA. The resulting single strand breaks (SSB) have been assumed to be repaired by the base excision (BER) pathway. Here we provide evidence that BER can also be a source of recombination events.

In *S.pombe*, the *rec12* gene (*SPO11* in other eukaryotes) is essential in the generation of meiotic double-strand breaks (DSB). Deletion of the *rec12* gene leads to loss of meiotic double-strand break (DSB) formation, reduces meiotic HR 100-1000-fold, and lowers spore viability to 20% (compared to 95% in *rec12*$^{+}$). The recombination frequency at *ade6* (M26 x 469) in homozygous *rec12*Δ crosses is 1.7ppm (prototrophs per million spores). When a glycosylase-deleted strain (*rec12*Δ*thp1*Δ*ung1*Δ) which accumulates uracil in DNA was crossed with a strain with intact glycosylases (*rec12*Δ*thp1*+*ung1*+), conversion at *ade6* increased to 10ppm, indicating that SSB formation induces meiotic homologous recombination (HR). Furthermore, expression of the human activation-induced deaminase (AID, which converts cytosine to uracil in DNA) in crosses homozygous for *rec12*Δ improved spore viability to 35%. This is consistent with an increase of crossover frequency, confirmed by crosses of *leu1*Δ x *arg4*Δ on chromosome II (2Mb distance). In *rec12*+ meiosis, the markers show no linkage, in *rec12*Δ meiosis, the frequency of wild-type recombinants was 0.051, and AID increased it to 0.093. In addition, in a cross homozygous for *ung1*Δ, the recombination frequency rose to 1.35, suggesting that Thp1 is more active in recombination initiation than Ung1.

We conclude that base excision repair (BER) can be a potential source of recombination. The molecular mechanism of this has yet to be defined.

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Program overview

CERLIB Meeting

Saturday, January 17

15h00: **Registration**

Monday, January 19

10h30-12h00: Pyrimidine Damage (*Chairman: Thierry Douki*)

10h30: **J. R. Wagner** *Free radical oxidation of cytosine and 5-methylcytosine: characterization of novel stable and unstable products*

11h00: **O. Brede** *Radical reactions in the system pyrimidine/aliphatic thiol*

11h20: **A. Banyasz** *Excited state relaxation and energy transfer in DNA double helices studied by femtosecond absorption spectroscopy*

11h40: **J. Wang** *A mechanistic study of the preferential photo-oxidation of the 5-CH_3 substituent of thymine and thymidine by $NH_4[VO(O_2)2(5\text{-}NO_2phen)]$*

12h00: Lunch & Free afternooon

17h00-18h50: Guanine Damage (*Chairman: Jean-Luc Ravanat*)

17h00: **C. J. Burrows** *Context effects on hydantoin formation and repair in DNA*

17h30: **N. E. Geacintov** *Reaction pathways of guanine radicals in solution and susceptibilities of removal of some of the stable reaction products by nucleotide excision repair mechanisms*

18h00: **G. Pratviel** *Guanine oxidation by high-valent metal-oxo porphyrins: electron transfer and oxygen atom transfer*

18h30: **V. Shafirovich** *Guanine lesions generated by oxyl radicals*

19h00: Welcome reception and dinner

Tuesday, January 20

8h30-9h50: Complex Lesions (*Chairman : Richard Wagner*)

8h30: **M. M. Greenberg** *Formation of DNA interstrand cross-links via oxidative stress*

9h00: **J.-L. Ravanat** *Complex DNA lesions generated subsequently to 2-deoxyribose oxidation*

9h30: **F. Bergeron** *Tandem lesions involving 8-oxo-purines in isolated DNA and polynucleotides: quantitation, mechanism of formation and repair*

10H40: Coffee break

10h20-12h00: Radiation-Induced DNA damage (*Chairman: Jean Cadet*)

10h20: **M. Sevilla** *Characterization of excitation induced hole transport and reactivity in DNA*

10h50: **C. Chatglialoglu** *Revising the reactions of hydroxyl radicals and hydrated electrons with guanine derivatives*

11h20: **M. Beuve** *Free radicals, oxygen effect and cell survival: an analysis by simulation*

11h40: **E. Dumont** *Chemical aspects of DNA damage: what can theoretical calculations tell us?*

12h00: Lunch & Free afternoon

17h00-19h10: DNA Lesions as Biomarkers (*Chairman: Alain Favier*)

17h00: **J. Cadet** *Oxidatively generated damage to DNA: measurement in cells*

17h30: **A. Collins** *Measurement of DNA damage and repair with the comet assay: recent developments*

18h00: **T. Douki** *HPLC-mass spectrometry analysis and Comet assay measurements of DNA damage induced by benzo[a]pyrene*

18h30: **M. Audebert** *Effect of Polycyclic Aromatic Hydrocarbons (PAHs) xenobiotics metabolism on DNA integrity, Development of a new genotoxic assay*

18h50: **S. Shaposhnikov** *Fluorescent in situ hybridisation on comets: Possible application to DNA damage and repair*

20h30: Poster Session

Wednesday, January 21

8h30-9h50: Oligonucleotides for Biochemical Studies (*Chairman, Dimitar Angelov*)

8h30: **S. Gambarelli** *Probing lesion-induced DNA conformational changes by pulsed EPR*

9h00: **Y. Wang** *Chemistry and biology of complex lesions induced by reactive oxygen species*

9h30: **M. Lomax** *Processing of clustered DNA damaged sites comprised of three lesions by the base excision repair pathway*

9h50 : Coffee break

10h20-12h00: DNA Repair (1) (*Chairman: Alain Sarazin*)

10h20: **D. Angelov** *Base excision repair within nucleosomal substrates*

10h50: **M. Atta** *DNA repair and free radicals: New insights into the mechanism of spore photoproduct lyase revealed by single mutation*

11h20: **J. Timmins** *Structure-function studies of DNA repair proteins from Deinococcus radiodurans*

11h40: **A.-S. Belmont** *Monitoring small molecules impact on cell repair capability using lesion containing oligonucleotide microarrays*

12h00: Lunch & Free afternoon

17h00-18h50: DNA Repair (2) (*Chairman: Peter O'Neill*)

17h00: **L. Mullenders** *Repair and biological effects of spontaneous and ionizing radiation induced DNA damage*

17h30: **A. Sarasin** *Transcription-coupled repair-deficient diseases: From Cockayne syndrome to UVSS syndrome*

18h00: **S. Sauvaigo** *Determination of the effects of aging and photoaging on DNA repair excision/synthesis capacities toward different DNA lesions using a functional biochip assay*

18h30: **A. Khobta** *Base excision repair protein Ogg1 contributes to impaired transcription at the sites of oxidative base damage*

20h30: Round Table Discussion "Tools for DNA Repair Quantification"

Organized by **S. Sauvaigo**

Thursday, January 22

8h30-9h50: DNA Polymerases (*Chairman: Evelyne Sage*)

8h30: **R. Fuchs** *Role of an Alkyltransferase-like protein in E. coli: the ybaZ gene product enhances Nucleotide Excision Repair of O6-alkylguanine adducts*

9h00: **C.-A. Reynaud** *Error-prone repair pathways in immunoglobulin gene hypermutation*

9h30: **G. Henneke** *Oxidative lesions processing by high-fidelity DNA polymerases in Pyrococcus abyssi*

9h40 : Coffee break

10h20-12h00: Cellular effects of oxidative stress to DNA (*Chairman Robert Fuchs*)

10h20: **E. Sage** *Repair of oxidized bases within multiply DNA damaged sites and mutation formation*

10h50: **Y. Saintigny** *Genetic Instability induced by tritium contamination*

11h20: **A. deGroot** *Deinococcus deserti: radiation tolerance and adaptation to harsh environmental conditions*

11h40: **V. De Rosa** *The anticancer effect of selenium: study of its role in DNA repair activity and resistance to UVA*

12h00: Lunch & Free afternoon

17h00-18h50: Oxidative DNA Damage and Human Diseases (*Chairman: Serge Cand*

17h00: **A. Favier** *Oxidative stress, DNA damage and male infertility*

17h30: **L. Hardie** *Oxidative DNA Damage in Barrett's Oesophagus*

18h00: **R. Olinski** *Possible link between oxidative DNA damage and cancer: i/ case of BRCA1 mutation carriers, ii/ relationship between DNA oxidation and methylation*

18h30: **T. Paz-Elizur** *Repair of oxidative DNA damage and the risk of tobacco-related cancers*

Friday, January 23

Breakfast and safe return home!


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