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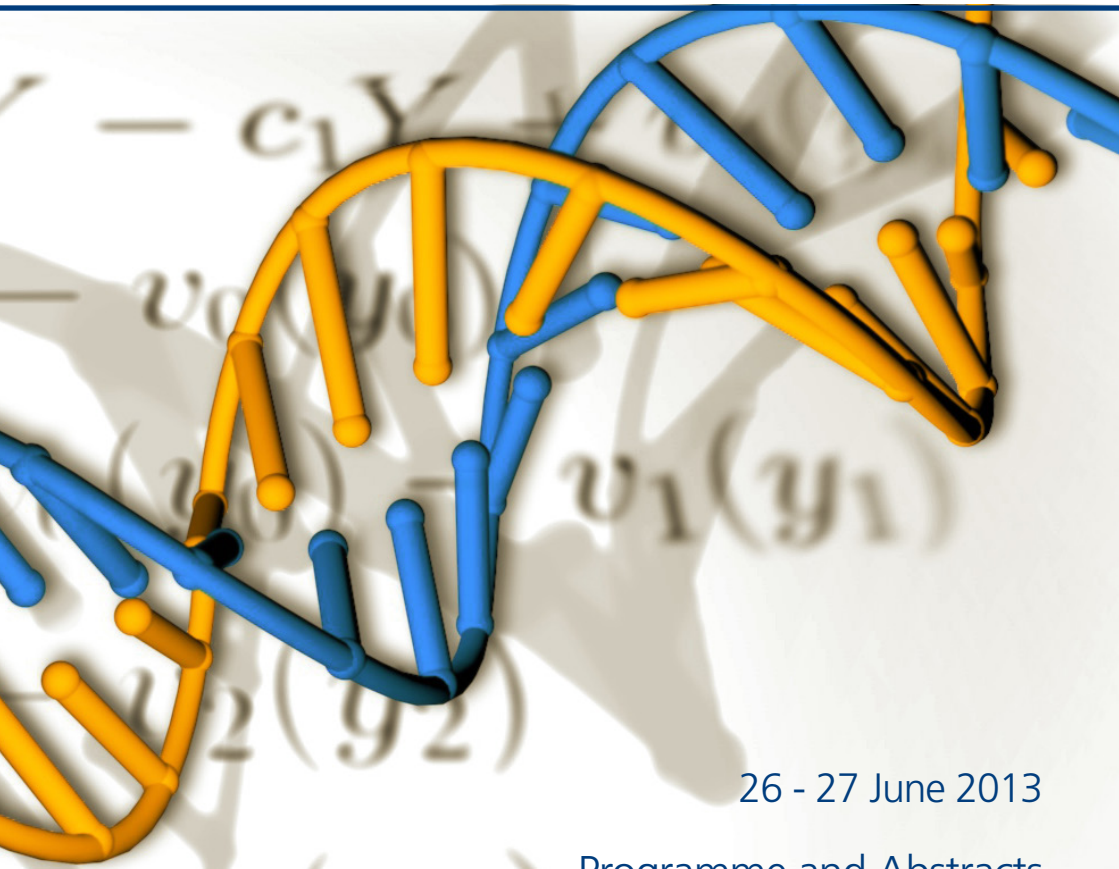
An international workshop

Mathematical Modelling of the DNA Damage Response



26 - 27 June 2013

Programme and Abstracts

MiLES Models and Mathematics in Life and Social Sciences

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Mathematical Modelling of the DNA Damage Response

Introduction

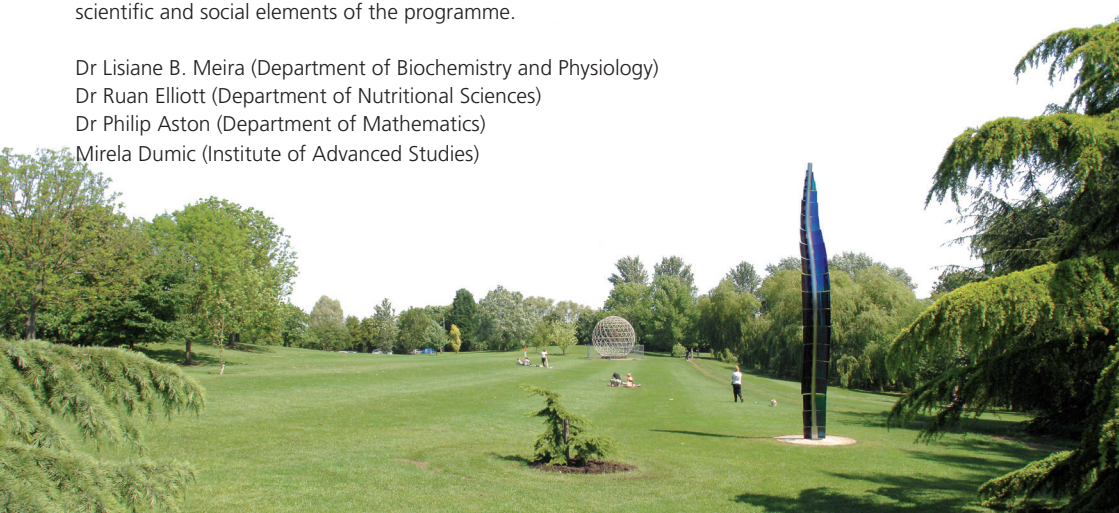
DNA damage is unavoidable. Intrinsic cellular metabolic processes as well as exposure to environmental, physical or chemical agents can lead to DNA damage. The ability to respond to unavoidable insults to our genetic material underpins human health and disease. A regulatory network of proteins has been identified that participates in DNA damage response (DDR). The importance of this network is clear from the existence of numerous human disorders in which DDR is defective. Significant progress has been made in understanding the mechanism of DNA repair pathways; however, multi-pathway dynamics are still inadequately described. Moreover, how the nature and/or abundance of DNA damage defines cell fate in specific cell types is still largely unknown.

In recent years, the use of computational and mathematical tools for addressing this important area of biology has become increasingly popular. However, there is growing recognition that there is a need for new mathematical tools to be developed allowing for the integration of these different networks in a way that mimics the complexity of the biological response, and at the same time for biologists to use a molecular/reductionist approach that can more effectively generate data that is suitable to inform the development of these new tools/models.

This two-day workshop will bring together biologists, mathematicians and computer scientists, and provide a highly interactive forum where the need for bridging the gap between experimentalists and modellers and recent advances in this exciting field are discussed. We are very grateful to acknowledge sponsorship from the Engineering and Physical Sciences Research Council (EPSRC), the University of Surrey's Institute of Advanced Studies (IAS) and the Faculties of Health and Medical Sciences (FHMS) as well as Engineering and Physical Sciences (FEPS).

We welcome you to Guildford and hope that you have an enjoyable and productive time in both the scientific and social elements of the programme.

Dr Lisiane B. Meira (Department of Biochemistry and Physiology)
Dr Ruan Elliott (Department of Nutritional Sciences)
Dr Philip Aston (Department of Mathematics)
Mirela Dumic (Institute of Advanced Studies)



Programme

Venue: School of Management, 32 MS 01

DAY 1

09:00	Registration and Coffee
0925	Welcome from Associate Dean for Research and Enterprise, FEPS Professor Karen Kirkby
0930	Opening remarks Lisiane B. Meira
SESSION 1	**DNA base damage and breaks**
Chair:	Lisiane B. Meira
0945	Leona D. Samson: "The pros and cons of DNA Repair"
1030	Sherif El-Khamisy: 'Breaking and sealing the human genome: the consequence of the imbalance'
1115	Coffee
1145	Philip Aston : "Mathematical modelling of base excision repair"
1230	Workshop group photo
1300	Lunch
SESSION 2	**Modelling repair and stress responses**
Chair:	Dragony Fu
1400	Kevin Janes: "Data-driven modeling of the cellular response to environmental insults"
1445	Peter Svensson: "Combining computational and experimental approaches to model genotoxic and mitotic stress responses"
1530	Coffee and posters
1630	Simon Reed: "How yeast global genome nucleotide excision repair is organized and orchestrated throughout the genome"
SHORT TALKS	
1715	Mark Bennett: "Novel bioinformatic tools for the analysis of DNA damage and repair processes throughout whole genomes"
1730	Renata Retkute: "DNA replication in cells exposed to DNA-damaging agents"
1800	Depart for dinner, Shere Village (transport organised)
2200	Return to Guildford

DAY 2

SESSION 3 DSB repair

Chair: Karen Kirkby

0930 **Filippo Rosselli:** "Origin, detection and repair of double-strand break in mammalian cells: a molecular symphony to ensure genome stability associated to a touch of genetic variation"

1015 **Norman Kirkby:** "Mathematical modelling: From DNA damage and repair to national demand for radiotherapy"

1100 Coffee

1130 **Yeyejide Adeleye:** "Pathway modelling of DNA damage and its application to risk assessment"

SHORT TALKS

1215 **Reza Taleei:** "Modelling DSB repair induced by ionizing radiation"

1230 **Adam Cole:** "Predicting badly behaved brain tumours with a MiNiMUS of fuss"

1245 Lunch

SESSION 4 Repair in the mitochondria

Chair: Ruan Elliott

1400 **Nadja Souza-Pinto:** "The nucleotide excision repair factor XPC modulates mitochondrial bioenergetics in human cells"

1445 **Dragony Fu:** "Human ALKBH7 protein plays a critical role in DNA damage-induced programmed necrosis"

1530 Coffee and posters

1600 - 1700 Workshop wrap-up and dissemination plan, **Ruan Elliott**

Speakers

The pros and cons of DNA repair
Leona D. Samson
Biological Engineering Department, Center for Environmental Health Sciences, MIT, USA

DNA damage has been clearly linked to a variety of human diseases, namely cancer, neurodegeneration and premature aging. We have studied the processing of DNA alkylation damage via several DNA repair pathways, including direct reversal pathways and base excision repair (BER). The biological role of the repair pathways has been studied in various mouse models. In particular we have examined the repair of DNA damage induced by model alkylating agents or by the reactive chemical species produced during an inflammatory response. In some circumstances the action of DNA repair can alleviate the detrimental effects induced by the DNA damaging agents, as one would normally expect, but in other circumstances the action of DNA repair can induce cell death and extensive tissue damage. The role of various DNA repair pathways in suppressing or causing disease will be discussed in the context of the large inter-individual differences in DNA repair capacity in human populations.

Bionote
Leona Samson is the Uncas and Helen Whitaker Professor, and an American Cancer Society Professor in the MIT Biological Engineering Department and the MIT Biology Department, as well as being a member of MIT's Koch Institute for Integrative Cancer Research and Center for Environmental Health Sciences. She has significant expertise in the study of DNA damage and repair, and has worked for over 30 years on the responses of yeast, rodent and human cells upon exposure to various DNA damaging agents found in exogenous and endogenous environments, as well as several DNA damaging agents used for cancer chemotherapy.

Breaking and Sealing the human genome: the consequence of the imbalance
Sherif El-Khamisy
MRC Genome Damage and Stability Centre, University of Sussex, UK

Defects in the repair of deoxyribonucleic acid (DNA) damage underpin several hereditary neurological diseases in humans. Of the different activities that repair chromosomal DNA breaks, defects in resolving damaged DNA termini in one strand of the double helix are among the most common causes of cell death. We will discuss how oxidative and topoisomerase-mediated DNA damage are repaired and their association with human disease. Particularly, we will focus on how enzymes that repair trapped topoisomerases, such as tyrosyl DNA phosphodiesterase 1 (TDP1) and the newly discovered enzyme TDP2, participate in protecting our genetic material from genotoxic stress.

Bionote
Sherif El-Khamisy has received his PhD in Biochemistry from the University of Sussex and after a post-doc

at St. Jude's Children Research Hospital, Tennessee, US has acted as a Wellcome Trust Fellow and Group Leader, in the Medical Centre for Genome Damage and Stability, University of Sussex, UK.

Mathematical modelling of base excision repair
Philip J. Aston, Ruan M. Elliott, Lisiane B. Meira
University of Surrey, UK

We first re-examine some data for base excision repair from the literature. Previously, in a mathematical model with estimated parameters, there was not good agreement between the model and the experimental data. However, we show that by fitting the model to the data, excellent agreement can be obtained. We also describe how the mathematical model can be simplified by considering two phases, a fast time phase through the initial stages of the repair process, and a slow time phase which is determined by the slowest step of the process. We then consider some new experimental data obtained for the early stages of the DNA repair process and show how the standard model can be made to fit part of the data, but not all of it. A modified model gives a good fit to all the data. We conclude with some reflections on interdisciplinary research involving mathematicians and biologists.

Bionote
Philip Aston is a Reader in the Department of Mathematics at the University of Surrey. He obtained his degree and PhD from Brunel University, before moving to Bath as a postdoc for 3 years. Then he became a lecturer at Surrey, a good few years ago! After 3 years as Director of Studies and 5 years as Head of Department, he started getting involved with research in more biological areas, which has included a mathematical analysis of models in pharmacokinetics/pharmacodynamics. He obtained a MILES Discipline-Hopping Fellowship to work with Lisi Meira and Ruan Elliott on DNA repair in a collaboration that is still continuing.

Data-driven modeling of the cellular response to environmental insults
Kevin A. Janes
Department of Biomedical Engineering, University of Virginia, USA

Cells possess a diverse array of sensors for environmental stresses, which transduce signals through a common set of effector pathways leading to cellular adaptation. Often, the relevant sensors and transducers are known for a particular environmental trigger, but their precise coordination during adaptation is unclear. In this talk, I will discuss computational approaches that use statistical regression of measured sensors and transducers to build predictive models of the adaptation response. Such "data-driven" models can identify jointly regulated pathways and suggest novel pathways when carefully analyzed. Our specific applications deal with a variety of death-inducing stimuli, but the methods are known to generalize to virtually any environmental input.

Speakers

Bionote
Kevin Janes is an assistant Professor of Biomedical Engineering at the University of Virginia, having obtained his BSc in Biomedical Engineering from Johns Hopkins University and a PhD in Bioengineering from the Massachusetts Institute of Technology. Kevin's lab is interested in new experimental and computational approaches for analyzing networks and answering network-level questions about signal transduction and gene expression.

H2Bub1 in genotoxic and mitotic stress responses
Laia Sadeghi, Lee Siggens, Karl Ekwall, ***Peter Svensson***
Karolinska Institutet, Department of Biosciences and Nutrition, Sweden

For computational analysis of genome-wide RNA expression and chromatin immunoprecipitation (ChIP) data, we have developed "Podbat". This software is dedicated to the analysis of transcription and protein occupancy over the genome of model organisms. We have generated genome-wide data to study the role of monoubiquitinated histone H2B (H2Bub1) after genotoxic and mitotic stress. Generally, H2Bub1 levels are correlated with transcription but H2Bub1 also plays a role in replication and differentiation as well as DNA damage responses. Following cellular exposure to DNA damaging agents, Podbat analysis revealed a slightly but significantly altered genome-wide distribution of H2Bub1, consistent with H2B ubiquitination in chromatin adjacent to the lesion. In addition to this direct function in the DNA damage response, we speculated that H2Bub1 might have an indirect role. Previous reports have implicated H2Bub1 in maintaining the borders between euchromatin and heterochromatin, leading us to map the genome-wide levels of heterochromatin mark H3K9me2 in H2Bub1-deficient cells. Interestingly, the most affected regions were the centromeres, chromosome regions that usually are not associated with H2Bub1. Through a series of experiments we identified a role for H2Bub1 in the setup of both yeast and human centromeres. In summary, the tumorigenic effects observed when reducing the levels of H2Bub1 in mammalian cells may be caused by a combination of improper signaling of DNA lesions and chromosome segregation defects caused by centromere dysfunction.

Bionote
Peter Svensson completed his PhD jointly at the Leiden University Medical Center, the Netherlands and Uppsala University, Sweden, in 2006. He did a post-doctoral period in the lab of Leona Samson at Massachusetts Institute of Technology, USA until 2009, after which he started his own group at Karolinska Institutet, Sweden.

How yeast global genome nucleotide excision repair is organized and orchestrated throughout the genome

Shirong Yu, Mark Bennett, Katie Evans, ***Simon Reed***
Department of Medical Genetics, Haematology and Pathology, School of Medicine, Cardiff University, UK

In response to UV radiation induced DNA damage, increased histone H3 acetylation at lysine 9 and 14 correlates with changes in chromatin structure and these alterations are associated with efficient global genome nucleotide excision repair (GG-NER) in yeast. GG-NER requires the activity of a heterotrimeric complex of Rad7, Rad16 and Abf1. We have previously shown that the GG-NER complex has E3 ubiquitin ligase activity and DNA translocase activity associated with the C3HC4 RING domain and ATPase domains of the Rad16 protein; both functions are necessary for efficient GG-NER. We have recently shown that UV induced histone H3 acetylation is regulated during GG-NER, and this activity promotes chromatin remodeling necessary for efficient repair of DNA damage. Our studies revealed that yeast Rad7 and Rad16 proteins drive UV induced chromatin remodelling by controlling histone H3 acetylation levels in chromatin. This is achieved via the concerted action of the ATPase, and C3HC4 RING E3 ligase domains of Rad16, which in concert regulate the occupancy of histone acetyl transferases such as GCN5 on chromatin in response to UV damage. Here we reveal how the ABF1 component of the GG-NER complex organizes GG-NER throughout the yeast genome. Using ChIP on chip, we have studied how UV induced changes in GG-NER complex binding initiated at ABF1 binding sites located throughout the yeast genome controls and orchestrates HAT occupancy, histone H3 acetylation status and DNA repair in response to DNA damage. These studies are providing remarkable insight not only into the mechanism of GG-NER but how nucleotide excision repair is organized in the genome.

Bionote

Dr Simon Reed is a Reader in Cancer Studies in the School of Medicine at Cardiff University. Reed gained a first class honours degree in Genetics in 1991, and a PhD in Molecular Biology in 1995, both from the University of Wales. He undertook his postdoctoral research as Fulbright Scholar in the Pathology Department at the University of Texas, USA. He established his research group in the UK following receipt of his first MRC Career Award becoming a senior lecturer at the School of Medicine at Cardiff University in 2003. He is the current chair of the UK Genome Stability Network, and is a member of the British Fulbright Scholars Association. In 2009 Reed received an AICR Cancer Researcher Award sponsored by Cell Press for his work on how nucleotide repair functions in chromatin.

Speakers

Origin, detection and repair of double-strand breaks in mammalian cells: a molecular symphony to ensure genome stability associated to a touch of genetic variation

Filippo Rosselli
UMR8200 CNRS Institut Gustave Roussy, France

DNA double-strand breaks (DSBs) are lesions arising from both cell-programmed and pathologic conditions. DSBs defective or incomplete repair result in genome rearrangements, senescence and/or cell death and is a major driver in developmental, neurological and immunological diseases as well as in cancer. DSBs are cell-programmed in two physiological conditions: during meiosis, to generate a different allele combination, to that observed in parents, and during immunoglobulin gene rearrangement, to ensure the high specificity and multiplicity of the immune response. Exposure to ionizing radiations and the collapse of stalled replication forks, as a consequence of the presence of road-blocking DNA lesions or sequences difficult to replicate, are the two major sources of "pathological" induced DSBs.
To restore the integrity of a broken DNA molecule, with the eventual loss of a minimal but safe (and necessary) dose of genetic instability, mammalian cells possess tens of proteins involved in recognition, signalling and repair of DSBs organized in multiple layers needing a highly and fine tuned spatiotemporal regulation. Noteworthy, to resolve DSBs, organisms have evolved two mechanisms: the non homologous end joining (NHEJ) and the homologous recombination (HR) with their variations. Indeed, the choice between the two pathways is of major importance to perform a quantitatively and qualitatively efficient DSBs repair, avoiding genome instability and diseases. We will strive to integrate the actual molecular knowledge from DSBs formation and repair to their consequences on cellular and organismal behaviour with a particular focus on the molecular and biochemical mechanisms involved in the DNA repair pathway choice.

Bionote
After obtaining his PhD in his native Italy, Filippo Rosselli joined the laboratory of Ethel Moustacchi as a post-doctoral fellow at the Institut Curie, France to study DSB repair and Fanconi anemia. He is now a group leader in the Gustave Roussy Cancer Institute in France. Filippo is a world leader in the field of DSB repair.

Mathematical Modelling: From DNA Damage and Repair to National Demand for Radiotherapy

Norman Kirkby
Department of Chemical & Process Engineering, University of Surrey

Multi-scale mathematical modelling is the obvious framework within which to capture the mechanisms and consequences of DNA damage and repair at various length scales from the cell, to tissue, to organ, to organism. In this talk a connected sequence of models will be presented briefly which show how understanding DNA damage and repair is fundamental. Attempts to connect these length scales also reveal absences in current knowledge.

The approaches taken will all be based around the example of glioblastoma, except at the largest length scales where a model of national demand for radiotherapy will be described briefly.

Bionote
Norman Kirkby is Professor of Chemical Engineering at the University of Surrey. Norman has obtained his BSc in Chemical Engineering from the University of Nottingham and a PhD in Chemical Engineering from the University of Cambridge. Norman's research is on systems biology and mathematical modelling of radiotherapy and cancer.

Pathway modelling of DNA damage and its application to risk assessment
Yeyejide Adeleye
Unilever Safety and Environmental Assurance Centre (SEAC), http://tt21c.org/, UK

The US National Academy of Sciences/National Research Council report on Toxicity Testing in the 21ST Century (Krewski et al 2010) proposes an approach to toxicity testing, focusing on toxicity pathways, which could ultimately negate the requirement to generate hazard data in animals for risk assessment. Key elements of the TT21C vision include defining exposure to chemicals, development of high content in vitro assays in human cell lines, dose response assessments, computational models of toxicity pathways and pharmacokinetic models supporting in vitro to in vivo extrapolation.
Toxicity pathways are defined as cellular response pathways that, when sufficiently perturbed, are expected to result in an adverse health effect. Biological processes are complex and in order to understand (and predict) toxicological responses it is essential to understand and determine the structure of pathways and networks. The structure and parameters of these pathways will determine their dynamics which will enable understanding of higher order cellular functions. Chosen for its known relationship to the development of human cancer, using the p53 pathway as a case study, our aim is to understand the complex interactions required to elicit adverse effects and using this knowledge to build computational models that allow adverse effects to be predicted from changes in specific biomarkers.

Bionote
Yeyejide Adeleye is a Computational Toxicologist with over 9 years experience in Bioinformatics and Systems Biology. As part of Unilever's ongoing effort to develop novel ways of delivering consumer safety without the use of animals, Ms Adeleye is involved in and leads projects that use Systems Biology (especially network and pathway analysis) to investigate the biological mechanisms of toxicological end points. Her current focus is the computational modelling of toxicity pathways and their application to risk assessments. She has been and invited speaker at various conferences and lecturer at the University of Surrey and Cranfield University. Ms Adeleye has sat on the Board of Directors for Cytoscape (a Systems Biology Platform) and also acts as a Cosmetics Europe Personal Care Association (formally COLIPA) Scientific Advisor on a FP7 Research Initiative Project to accelerate the development of non-animal test methods.

Speakers

The nucleotide excision repair factor XPC modulates mitochondrial bioenergetics in human cells

Nadja C. de Souza Pinto
Department of Biochemistry, Chemistry Institute, University of São Paulo, SP, Brazil

Mitochondrial integrity is essential for cellular homeostasis, as mitochondria play pivotal roles in general metabolism, energy production and cellular fate after stress. Mitochondria contain their own genome (mtDNA), which encodes for 13 essential subunits of 4 of the 5 oxidative phosphorylation complexes. As the mtDNA is located near the electron transport chain, it is a major site for oxidation by the reactive oxygen species generated as by-products of respiration, and, in fact, it accumulates higher levels of oxidized bases than the nuclear DNA. The base excision repair pathway has been thoroughly characterized in mitochondria, but the presence of other repair pathways is still unclear. The nucleotide excision repair (NER) pathway, which deals with bulky, helix-distorting lesions, is highly conserved from bacteria to higher eukaryotes. NER is initiated by lesion recognition by one of two independent mechanisms, RNA polymerase stalling at the lesion site in transcription-coupled repair, or XPC binding in global-genome repair. This pathway is essential for genome surveillance, and its absence results in severe human syndromes such as Xeroderma pigmentosum, Cockayne syndrome and Trichothiodystrophy. Nevertheless, despite mitochondrial localization of some NER factors, canonical NER has not been found in mammalian mitochondria. But since the XPC protein was found to stimulate repair of oxidized bases via a functional interaction with the Oxoguanine DNA glycosylase (OGG1), and this protein is essential for 8-hydroxiguanine repair in mtDNA, we investigated whether XPC played a role in mtDNA maintenance and mitochondrial integrity. Surprisingly, we found a significant decrease in mitochondrial function in human fibroblasts, XP4PA, expressing a mutated XPC (which is unstable resulting in a functional knockout) when compared with normal fibroblasts, MRC5. Mitochondria from the XPC cells showed a significant lower respiratory capacity, maximum respiration and lower calcium uptake. On the other hand, hydrogen peroxide and superoxide anion production were significant elevated when compared to the normal cells. And while mtDNA copy number was not reduced in the XPC cells, PGC-1α mRNA levels were virtually undetected. Together these results suggested that XPC deficiency results in a severe bioenergetic defect, likely due to altered transcription of mitochondrial proteins. We are now investigating whether this is associated with accumulation of mtDNA damage due to a direct role of XPC in stimulating OGG1-initiated mtDNA repair. Our results suggest a new role for the XPC protein in controlling cellular metabolism.

Bionote

Nadja Souza Pinto obtained her PhD in Molecular Biology at the Universidade de São Paulo (USP), Brazil. After a long post-doc at the National Institute of Aging, Nadja is now Professor at the Chemistry Institute in the Universidade de São Paulo. Nadja is an associated editor for "Mechanisms of Ageing and Development". Nadja is a world leader in the field of mitochondrial DNA repair and bioenergetics.

Human ALKBH7 protein plays a critical role in DNA damage-induced programmed necrosis

Dragony Fu
Institute of Veterinary Biochemistry and Molecular Biology, University of Zurich, Switzerland

Programmed necrosis represents an emergent form of cell death that is induced in response to several forms of cellular stress, including genotoxic DNA damage. Here, we show that the human AlkB homolog 7 (ALKBH7) protein triggers the collapse of mitochondrial membrane potential and loss of mitochondrial function that lead to energy depletion and programmed necrotic cell death. Depletion of ALKBH7 suppresses necrotic cell death induced by numerous alkylating and oxidizing agents by maintaining their mitochondrial membrane potential. Furthermore, we show that programmed cell death mediated by ALKBH7 is important for eliminating cells that have accumulated irreparable amounts of genomic DNA damage, thereby serving as a mechanism to eliminate cells that have chromosomal damage that can lead to mutagenesis and therefore cancer development. Thus, ALKBH7 represents a critical factor in determining the cell fate decision to survive or die after DNA damage.

Bionote
Dragony Fu completed his Ph.D. thesis at the University of California Berkeley, USA, in the laboratory of Kathleen Collins. He then became a postdoctoral fellow in the laboratory of Leona D. Samson at the Massachusetts Institute of Technology, Cambridge, USA, where he discovered novel cellular functions for mammalian ALKBH dioxygenases in nucleic acid modification and the genotoxic stress response. Currently, Dr. Fu is a visiting research fellow at the University of Zurich where he is continuing his research on the ALKBH enzymes and their diverse biological roles.

Short talks/Posters

Novel bioinformatic tools for the analysis of DNA damage and repair processes throughout whole genomes

Mark Bennett, Department: Institute of Cancer and Genetics, Cardiff University, UK

We have developed a technology to measure DNA damage at high resolutions throughout whole genomes (Teng et al 2011). Based on ChIP-chip (chromatin immunoprecipitation combined with microarray chips), the technology allows a measurement of varying damage levels across the genome and, by measuring this damage at time points after damage induction, a measure of varying repair rates. Combining the data with conventional ChIP-chip – measuring factors associated with DNA damage and repair, such the binding locations of DNA repair proteins and epigenetic modifications on histones – allows multiple aspects of the DNA damage response and subsequent repair process to be examined genome-wide, allowing us to begin to piece together how the process acts at individual sites in the genome in the complex environment of chromatin. A model to predict sequence specific damage profiles has been created, based on genome sequence, which has allowed us to test the validity of this technology. ChIP-chip produces large amounts of data and so requires bioinformatic tools for its analysis. Several such tools have previously been created, but are too limited for the type of analyses we need to undertake with the data we generate. A selection of novel bioinformatic tools have therefore been developed to perform these analyses. The most important of these is a novel inter-dataset normalisation procedure, which allows comparisons to be made between datasets from different conditions. Previously, analyses were limited to determining whether or not the presence or absence of factor changed between conditions, that is, whether or not the presence of the factor at a given site was gained or lost. We are now able to determine changes in the levels of the factor at sites where the binding state itself does not change. This opens up a whole new dimension of analyses for ChIP-chip data, far beyond the work presented here. It is allowing us to analyse DNA damage and its repair, in the context of associated cellular factors, to determine the relationships between them at high resolutions across whole genomes. These data are beginning to allow us to piece together the interactions of the factors involved in DNA repair in the context of chromatin, thereby adding to our understanding of the mechanisms of this process.

Y. Teng, M. Bennett, K.E. Evans, H. Zhuang-Jackson, A. Higgs, S.H. Reed, and R. Waters. A novel method for the genome-wide high resolution analysis of DNA damage. Nucleic Acids Research, 39(2):e10, 2011.

DNA replication in cells exposed to DNA-damaging agent

Renata Retkute, Department: School of Biosciences, University of Nottingham, UK

We introduce a mathematical model for DNA replication kinetics in the presence of defects resulting from DNA damage. DNA replication, the process during which cells' genetic information is duplicated, is one of the most fundamental processes in biology. Eukaryotic DNA replication is initiated from multiple sites (replication origins) on the chromosome by assembling bidirectional complexes called replication forks. In cells exposed to DNA-damaging agent the DNA damage has the inhibitory effect on replication in

eukaryotic cells: damage in the leading strand may block the entire process of replication fork progression, but replication can be rescued by activation of dormant replication origin.

Modelling DSB repair induced by ionizing radiation
Reza Taleei, Oncology Pathology, Karolinska Institutet, Sweden

Ionizing radiation induces a variety of damage to DNA, including single strand breaks (SSB), base lesions (BL) and double strand breaks (DSB). DSB is the most toxic damage induced by ionizing radiation. The aim of this work is to propose a mathematical model that explains DSB repair processes. Three main DSB repair pathways are nonhomologous end-joining (NHEJ), homologous recombination (HR), and microhomology-mediated end-joining (MMEJ). NHEJ is the predominant repair pathway in mammalian cells through the whole cell cycle. HR and MMEJ are involved in the repair of DSB during late S and G2 phases, and G1 phase of the cell cycle, respectively. The NHEJ pathway is a fast repair process with the repair half time of less than 30 minutes. MMEJ and HR repair pathways require resection to start the repair in a slow process. Complex type DSB is defined as DSB in close proximity of at least one strand break. The complexity of the DSB increases with LET. It is assumed that complex DSB require further end processing (such as resection) because the actual binding site of core NHEJ repair proteins is about 2 or 3 helical turns and presence of another SSB impairs repair activity of NHEJ repair proteins. Beside the local complexity of DSB ends, repair of damage in the heterochromatin is observed to take longer. We have developed a computational mechanistic model of DSB-repair including the three main pathways in a cell cycle dependent manner. In G1 and early S phases of the cell cycle the repair pathways NHEJ and MMEJ are active, while in G2 and late S, the NHEJ and HR pathways remain active. Repair of DSB predominantly starts with NHEJ and completes if the DSB is a simple type, while complex DSBs are repaired with a second option of MMEJ in G1 and early S, and HR in G2 and late S phases. We have assumed that 15% of the damage is in the heterochromatin and the repair of the damage in the heterochromatin involves further molecular processes that relax the heterochromatin which delays the repair. To test the DSB-repair model, simulated DSBs induced by single tracks of ionizing radiation were subjected to repair schemes as described in our model. The DSB-repair kinetic curve calculated by the solution of the model is in good agreement with the measured repair kinetics for human cells irradiated with ions of different LETs. The results confirm that the complex type DSB is repaired with slow repair kinetics. The complexity of DSB is a unique feature of DSB that has been used to predict the repair kinetics of DSB for radiation exposure of different quality.

Predicting badly behaved brain tumours with a MiNiMUS of fuss
Adam Cole, Department: Chemical Engineering, University of Surrey, UK

The Medical Applications (of Ion Beams) Group based at Surrey are a multidisciplinary group that focus on the basic in vitro radiobiology that underpins radiotherapy with a particular interest in treatments for GBM cell lines. The main aim of this project is to form a mathematical model to predict the formation and numbers of micronuclei (MN). The model has colloquially become known as MiNiMUS (MIcroNucleI

Modelling at the University of Surrey). The model builds on a correlation of the numbers of double strand breaks (DSBs) caused by ionising radiation to the numbers of MN found in a cohort of cells. In addition to this the model considers two main pathways for DSB repair, Homologous Recombination (HR) and Non Homologous End Joining (NHEJ). At the heart of the model is a binary decision tree. Each DSB from a treatment is fired through the tree and the outcome recorded. The probability at each node is determined by a combination of the phase of the cell cycle that the treatment was done in and biologically relevant experiments. Overlaying this model are Monte Carlo sampling routines to account for inherent difficulties of predicting cohort behaviour from singular cell responses. Any probability used in the model is considered the mean of a Beta distribution. A new mean is sampled using acceptance/rejection testing. Currently the model predicts experimental data from literature for peripheral blood lymphocytes as these are better characterised, but experiments with GBM cell lines are ongoing. The experimental procedure uses flow cytometry and a double DNA staining technique to calculate the number of micronuclei from cohorts of irradiated cells. The model has been set up so that it can be used as a framework for future development of the individual probabilities in the tree. It is the intention to implement more deterministic models of DNA repair probabilities rather than stochastic modelling.

Elucidating the mechanism of Aag-dependent cell death

Fahad Alhumaydhi, Department: Biochemistry and Physiology, University of Surrey, UK

DNA damage results from many endogenous and environmental agents such as alkylating agents. Base excision repair (BER) is key for the repair of DNA bases modified by alkylating agents. Initiation of the BER pathway occurs via the removal of a damaged alkylated base by an enzyme called alkyladenine DNA glycosylase (AAG). AAG excises alkylated DNA bases in the BER pathway generating abasic sites (AP sites) within the DNA which are processed to form single strand breaks (SSBs). These BER intermediates (AP sites and SSBs) are very toxic to the cell. Any imbalance of the BER pathway can lead to accumulation of these AP sites and SSBs and potentially trigger cell death via hyperactivation of poly (ADP-ribose) polymerase 1 (PARP1). In our study, Aag proficient and deficient cells were treated with an alkylating agent. The temporal changes in AP site number and cell death incidence were characterised. The results show that the kinetics and amount of AP site formation is different in Aag proficient versus Aag deficient cells. Increased AP site formation correlates with increased cell death in Aag proficient cells while Aag deficiency protects cells from alkylation-induced cell death, at least in earlier time points. These results will be used to build a mathematical model to explain AAG-mediated alkylation-induced cell death.

The nuclear morphometry analysis (NMA) as a tool for evaluation of senescence in human fibroblasts deficient in Nucleotide Excision Repair

Larissa Milano de Souza, Biophysics, University of Rio Grande do Sul, Porto Alegre, Brazil

Milano, L.1 ; Guecheva, T.1 ;FilippiChielaE.2; Lenz G.2; Saffi J.3
1 Laboratory of "Eukaryotic DNA Repair", Biophysics Department/Center of Biotechnology, Federal University of Rio Grande do Sul, Porto Alegre, Brazil

2 Laboratory of " Cell Signaling and Plasticity", Biophysics Department/Center of Biotechnology, Federal University of Rio Grande do Sul, Porto Alegre, Brazil
3 Laboratory of "Genetic Toxicology" Department of Basic Health Sciences, UFCSPA, Porto Alegre, Brazil

The mechanisms of action of the anthracyclines doxorubicin (DOX) and daunorubicin (DNR) include topoisomerase II-poisoning, free radicals release and DNA adducts formation. This can trigger a cell cycle arrest, which allows the cell to recruit DNA repair proteins, senescence or cell death induction. The nucleotide excision repair (NER) participates in the removal of lesions that distort the double helix of DNA. Cell lines deficient in NER are more sensitive to anthracyclines, however little is known about the cellular processes that permit cell survival after treatment with these drugs. In this work we verified the cellular senescence induced by DOX and DNR in human fibroblasts proficient (MRC5) and deficient (XPA) in NER. For this propose we developed a tool based in nuclear morphometry analysis (NMA) in order to quantify senescence using Image J Software. As a standard methodology we performed senescence-associated (beta)galactosidase (SA-beta-gal) assay. An increased percentage of SA-beta-gal cells at concentration of 0.05 ug/ml DOX and DNR was observed in MRC5 and XPA cells (about 70% and 50%, respectively) indicating senescence. The same pattern was found using the nuclear size as analysed with NMA. Although preliminary, these results demonstrate that the NMA is an adequate method for analysis of cellular senescence.

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Regulation of meiotic DNA double-strand break formation by the DNA damage checkpoint kinases Mec1(ATR) and Tel1(ATM)

Matthew J Neale, Valerie Garcia, Steve Gray, Rachal Allison, MRC Genome Damage and Stability Centre, School of LIfe Sciences, University of Sussex

During gametogenesis, high levels of DNA double-strand breaks are created and repaired throughout meiotic prophase to drive interaction between homologous chromosomes and—through recombination—generate genetically diverse gametes. In our laboratory we use the model eukaryote, S. cerevisiae to investigate the molecular mechanisms underpinning meiotic DSB formation, DSB end-processing, ssDNA resection and DSB repair. Understanding and modeling these processes is complex: in each cell approximately 200 DSB events are dispersed across the four copies of all chromosomes (two homologues each with two sister chromatids) in a non-random distribution that is influenced by various levels of chromatin organisation. Methods of analysis span site-specific assays for DSB formation and repair through to genome-wide measures of recombination using pulsed-field gel electrophoresis, hybrid genetic crosses, and next-generation sequencing of recombination intermediates. Our current work investigates how activation of the DNA damage response by the evolutionarily conserved checkpoint kinases ATM and ATR influences the frequency, distribution, and repair outcome of DSB events. An ability to mathematically model, to make predictions, and to test them in our genetically tractable system would be of great value to our research field.

List of Participants

Yeyejide Adeleye
Unilever Safety and Environmental Assurance Centre (SEAC), http://tt21c.org/, UK
yeyejide.adeleye@unilever.com

Fahad Alhumaydhi
Department: Biochemistry and Physiology, University of Surrey, UK
fa00065@surrey.ac.uk

Abdullah Aljohani
University of Surrey, UK
aa00148@surrey.ac.uk

Philip J. Aston
University of Surrey, UK
p.aston@surrey.ac.uk

Lara Barazzuol
University of Sussex, UK
l.barazzuol@sussex.ac.uk

Mark Bennett
Department: Institute of Cancer and Genetics, Cardiff University, UK
bennettmr1@cf.ac.uk

Adam Cole
Department: Chemical Engineering, University of Surrey, UK
a.cole@surrey.ac.uk

Ruan Elliott
Department of Nutritional Sciences, University of Surrey, UK
r.elliott@surrey.ac.uk

Clara Forrer Charlier
University of Surrey, UK
clara.forrer.charlier@gmail.com

Dragony Fu
Institute of Veterinary Biochemistry and Molecular Biology, University of Zurich, Switzerland
dragony@mit.edu

Kevin A. Janes
Department of Biomedical Engineering University of Virginia, USA
kaj5f@eservices.virginia.edu

Charlie Jeynes
Ion Beam Centre,University of Surrey, UK
j.c.jeynes@surrey.ac.uk

Sherif El-Khamisy
MRC Genome Damage and Stability Centre, University of Sussex, UK
s.f.el-khamisy@sussex.ac.uk

Norman Kirkby
Department of Chemical and Process Engineering, University of Surrey, UK
n.kirkby@surrey.ac.uk

Karen Kirkby
Ion Beam Centre, University of Surrey, UK
k.kirkby@surrey.ac.uk

Lisiane Meira
Department of Biochemistry and Physiology, University of Surrey, UK
l.meira@surrey.ac.uk

Mike Merchant
Ion Beam Centre, University of Surrey, UK
m.merchant@surrey.ac.uk

List of Participants

Larissa Milano de Souza
Department of Biophysics
University of Rio Grande do Sul, Porto Alegre, Brazil
milano.lari@gmail.com

Ali Jbbar
Department of Chemistry and Physiology
University of Surrey, UK
a.jbbar@surrey.ac.uk

Matthew Neale
University of Sussex, UK
m.neale@sussex.ac.uk

Renata Retkute
Department: School of Biosciences
University of Nottingham, UK
renata.retkute@nottingham.ac.uk

Simon Reed
Department of Medical Genetics, Haematology and Pathology, School of Medicine, Cardiff University, UK
simonhreed@mac.com

Filippo Rosselli
CNRS Institut Gustave Roussy, France
rosselli@igr.fr

Leona D. Samson
Biological Engineering Department, Center for Environmental Health Sciences, MIT, USA
lsamson@mit.edu

Nadja C. de Souza Pinto
Department of Biochemistry, Chemistry Institute, University of São Paulo, SP, Brazil
nadja@iq.usp.br

Peter Svensson
Karolinska Institutet, Department of Biosciences and Nutrition, Sweden
peter.svensson@ki.se

Reza Taleei
Oncology Pathology, Karolinska Institutet, Sweden
reza.taleei@ki.se

Dan Whelligan
Department of Chemistry
University of Surrey, UK
d.whelligan@surrey.ac.uk

Department of Biochemistry and Physiology (FHMS)
Department of Nutritional Sciences (FHMS)
Department of Mathematics (FEPS)

University of Surrey
Guildford, Surrey GU2 7XH UK

www.surrey.ac.uk

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