Life Science Project Titles 2011-2012

Genome, Damage and Stability Subject Area

Life Science Projects 2011-2012

Faculty Name: Dr. Jon Baxter
Room No: G3.05 **Email:**Jon.Baxter@sussex.ac.uk

Project Title/Area:
Investigating the genetic relationship between SMC/Condensin and topoisomerases in the yeast *Saccharomyces cerevisiae* / Genome

Course requirements:	**No of places:** 1

Further Information:

Recently we have demonstrated that SMC/Condensin is required in vivo to promote supercoiled structures that are rapidly resolved by topoisomerase II. This project will use yeast genetics to study the genetic interactions between hypomorphic SMC/condensin mutants with mutants of each of the yeast topoisomerase genes. This project will clarify the mechanism of SMC/condensin in chromosome resolution.

Life Science Projects 2011-2012

Faculty Name: Dr. Alessandro Bianchi **Room No:** 2C37 **Email:** a.bianchi@sussex.ac.uk	
Project Title/Area: Isolation of mutations in the fission yeast telomeric protein Tpz1 affecting telomere length or telomere protection	
Course requirements:	**No of places:** 1
Further Information: We intend to conduct a random mutagenesis analysis of the fission yeast gene tpz1, in order to identify mutations that would offer insight into the mechanism of action of the protein in the regulation of telomere length and telomere protection. Mutations will be introduced in the gene on a plasmid, the plasmid will be introduced into fission yeast cells, then a phenotypic analysis will be carried out, and plasmids conferring interesting phenotypes will be extracted from yeast and then analysed by DNA sequencing. This will allow to map important domains in the gene and to gain insight into its complex function at telomeres.	
Project Title/Area: Mapping the domain(s) in the Rif1 protein required for interaction with the telomeric binding protein Taz1	
Course requirements:	**No of places:** 1
Further Information: A complex of at least three proteins in fission yeast is required to inhibit telomerase action at telomeres: Taz1 , Rap1 and Rif1. The interplay between these proteins is poorly characterised. We would like to begin to obtain information about the molecular architecture of this complex by determining the region(s) in the large Rif1 protein that are required for interaction with the telomeric DNA binding factor Taz1. These studies will be carried out using a classic molecular genetic assay, two-hybrid analysis, and will be a first step in the characterization of the complex function of telomeric protein Rif1. We will also attempt to determine whether Rap1 and Rif1 bind to the same region in Taz1, thus possibly competing with one another for binding at telomeres.	

Life Science Projects 2011-2012

<table>
<tr><td colspan="2">Faculty Name: Prof. Keith Caldecott
Room No: Email:k.w.caldecott@sussex.ac.uk</td></tr>
<tr><td colspan="2">Project Title/Area:

Expression and purification of human DNA repair proteins</td></tr>
<tr><td>Course requirements:
Should be enrolled on 3rd yr genetic disease and cancer module</td><td>No of places: 2</td></tr>
<tr><td colspan="2">Further Information:

Expression constructs encoding DNA repair genes implicated in human genetic disease will be expressed in E.coli and the recombinant proteins purified by affinity chromatography for biochemical analysis.</td></tr>
<tr><td colspan="2">Project Title/Area:

Link between mutations in DNA single-strand break repair/base excision repair genes and cancer incidence (dry project)</td></tr>
<tr><td>Course requirements:</td><td>No of places: 1</td></tr>
<tr><td colspan="2">Further Information:

The project will require detailed survey of the scientific literature (Pubmed etc) and/or public databases for evidence of mutations and/or deregulated expression of DNA single-strand break repair/base excision repair genes in cancer.</td></tr>
</table>

Life Science Projects 2011-2012

<table>
<tr><td colspan="2">Faculty Name: Prof. Tony Carr
Room No: G418 Email: a.m.carr@sussex.ac.uk</td></tr>
<tr><td colspan="2">Project Title/Area:</td></tr>
<tr><td>Course requirements: BioMed, Bichem</td><td>No of places: 2</td></tr>
<tr><td colspan="2">Further Information:
The project will create and/or use plasmid reagents and engineered yeast strains to makes site specific mutations in a specific DNA repair gene in the genome of the yeast S. pombe. The student will learn PCR, cloning, site-directed mutagenesis and yeast transformation. Stains in which mutations have been successfully introduced will be assayed by survival analysis for their ability to tolerate specific genotoxins.

A detailed appraisal of the methodology is available in the following publication. Gene. 2008 Jan 15;407(1-2):63-74: Gene tagging and gene replacement using recombinase-mediated cassette exchange in Schizosaccharomyces pombe. Watson AT, Garcia V, Bone N, Carr AM, Armstrong J.</td></tr>
</table>

Life Science Projects 2011-2012

Faculty Name: Prof. Aidan Doherty
Room No:G3-20 **Email:** ajd21@sussex.ac.uk

Project Title: Mutagenesis studies on DNA polymerases involved in DNA break repair

Course requirements: Enthusiastic student interested in DNA	**No of places:** 1

Further Information:

Failure to correctly repair DNA breaks can result in genome instability and cancer. We are studying the role of specific DNA polymerases in repairing double-strand breaks. We have elucidated a number of crystal structures of these enzymes bound to DNA, which has enabled us to identify residues potentially involved in DNA binding and repair. The project will involve mutating a number of these residues and studying the biochemical properties of these mutants with a view to identifying their specific function in break repair.

Potential techniques involved in the project are: Site directed mutagenesis; Protein expression and advanced protein purification (affinity chromatography, ion exchange chromatography, size exclusion chromatography); DNA binding assays (gel and spectrophotometric based), Enzymatic analysis (gel and spectrophotometric based); Biophysical analysis.

References:

1. Brissett, N.C., Martin, M.J., Pitcher, R.S., Green, A.J., Fox, G.C.,Blanco, L. & Doherty, A.J. (2011) Structure of a novel pre-ternary complex involving a prokaryotic NHEJ polymerase. Mol. Cell 41, 221-231.

2. Brissett, N.C., Pitcher, R.S. Picher, A.J., Andrade, P. Juarez, R., Green, A.J., Dafforn, T., D. Fox, G.C., Blanco, L. & Doherty, A.J. (2007) Structure of a polymerase-mediated DNA NHEJ synaptic complex. Science 318, 456-459.

3. Pitcher, R.S. Brissett, N.C. Picher, A.J., Andrade, P. Juarez, R., Thompson, D. Fox, G.C., Blanco, L. & Doherty, A.J. (2007) Structure and Function of a Mycobacterial NHEJ DNA repair polymerase. J. Mol. Biol. 66, 391–405.

Faculty Name: Prof. Aidan Doherty (continued)
Room No:G3-20 **Email:** ajd21@sussex.ac.uk

Project Title: Purification and characterisation of a DNA polymerase involved in mitochondrial DNA maintenance

Course requirements: Enthusiastic student interested in DNA and mitochondria	**No of places:** 1

Further Information:

Recent work from our laboratory has implicated a family of DNA polymerases in the maintenance of the mitochondrial genome in higher eukaryotes, including humans. Maintenance of mitochondrial DNA is an important (and yet poorly understood) process, with mitochondrial dysfunction being linked to a number of human diseases, including cancers and neurodegeneration. The project proposed here will aid in the understanding of this family of DNA polymerases, ultimately giving insight into mitochondrial DNA maintenance. The primary objective of this proposal is the purification of the polymerase, which will be an important tool for the laboratory in functional and structural studies. Techniques employed will be mutagenesis PCR (polymerase chain reaction), molecular cloning, protein expression and purification.

References:

1. Holt IJ (2010) Zen and the art of mitochondrial DNA maintenance.
Trends Genet. 26,103-9.

2. Falkenberg M, Larsson NG, Gustafsson CM. (2007) DNA replication and transcription in mammalian mitochondria. Annu Rev Biochem. 76, 679-99.

Project Title: Eukaryotic Mitochondrial DNA repair pathways

Course requirements: Enthusiastic student interested in DNA and mitochondria	**No of places:** 1

Further Information:
For many years, the repair of most damage in mitochondrial DNA (mt DNA) was thought limited to short-patch base excision repair (SP-BER), which replaces a single nucleotide by the sequential action of DNA glycosylases, an apurinic/apyrimidinic (AP) endonuclease, the mitochondrial DNA polymerase γ, an abasic lyase activity, and mitochondrial DNA ligase. However, the likely array of lesions inflicted on mt DNA by oxygen radicals and the possibility of replication errors and disruptions indicated that such a restricted repair repertoire would be inadequate. Recent studies have considerably expanded our knowledge of mtDNA repair to include long-patch base excision repair (LP-BER), mismatch repair, and homologous recombination and non homologous end-joining. This project will examine the recent literature in this fast moving field and review our current knowledge of DNA repair pathways known to operate in eukaryotic mitochondria.

References:

1. Liu, P. & Demple, B.(2010) DNA repair in mammalian mitochondria: Much more than we thought? Environ Mol Mutagen. 51, 417-26.

2.Holt, I.J. (2010) Zen and the art of mitochondrial DNA maintenance.
Trends Genet. 26,103-9.

Life Science Projects 2011-2012

Faculty Name: Dr. Jessica Downs
Room No: G4.12 **Email:** j.a.downs@sussex.ac.uk

Project Title/Area:
Analysis of the PBRM1 gene in human health and disease. A Critical Review Project.

Course requirements: Advanced Molecular Cell Biology. Bioinformatics and/or Proteins in Action	**No of places:** 1

Further Information:

PBRM1 is a tumour suppressor gene that encodes a protein termed BAF180 and plays a key role in preventing the development of cancer. It is a very large gene, and there are multiple alternative transcripts. This project will include a detailed compilation of data from a wide range of databases and literature sources in order to provide a comprehensive gene analysis, including information about expression and localization of PBRM1 transcripts in normal tissues, and mutations and misregulation of the gene that have been reported in cancer cells. These data will provide the basis for a critical analysis of PBRM1 function.

Project Title/Area:
Investigation into the INO80 chromatin remodelling complex in DNA damage responses

Course requirements:	**No of places:** 1

Further Information:

The INO80 chromatin remodelling complex is a large, multisubunit complex that reorganizes chromatin structure in cells. We have found that INO80 facilitates DNA damage responses in yeast, and recently, studies in human cells found that this activity is conserved. In this project, a combination of genetics and protein biochemistry approaches will be used to investigate the mechanism by which INO80 functions to facilitate DNA damage responses.

Life Science Projects 2011-2012

Faculty Name: Dr. Sherif El-Khamisy
Room No: GDSC G3.02 **Email:** smfame20@sussex.ac.uk

Project Title/Area: Neurodegeneration and DNA Strand Break Repair

Course requirements: None

No of places: 1

Further Information:

Oxidative stress is an etiological factor for several neurodegenerative disorders and is a major source of DNA single-strand breaks (SSBs), which are the commonest type of DNA damage arising in cells (tens of thousands per cell/per day). In addition, abortive activities of DNA topoisomerases leads to DNA breaks that have been associated with human ataxia (El-Khamisy et al., Nature, 434: 108-113, 2005). Cells have employed different mechanisms to repair such breaks, including the hydrolytic cleavage of the phosphodiester bond between the stalled topoisomerase and DNA. The prototype enzyme for such an activity is TDP1, which is also involved in the repair of a variety of oxidative 3'-termini and mutation of which is associated with the neurological disorder SCAN1. Despite this important catalytic function fulfilled by TDP1, it remains together with the newly identified enzyme TDP2 (Ledesma et al., Nature, 461:674-8) the only known human enzymes that display this activity. Here, we will identify novel regulatory mechanisms for the repair of DNA topoisomerase-mediated and oxidative stress-induced DNA breaks, using a combined approach of yeast genetics and biochemical analysis.

For further information please visit: http://www.sussex.ac.uk/gdsc/profile143781.html

Life Science Projects 2011-2012

Faculty Name: Dr. Helfrid Hochegger
Room No: G3.09 **Email:** hh65@sussex.ac.uk

Project Title/Area:
Proteomic analysis of chromatin binding factors regulated by Cdk activity

Course requirements:	**No of places:** 2
High motivation and good laboratory skills	

Further Information:
Cdks control S-phase progression by triggering DNA replication and fine tuning DNA repair pathways. These steps are crucial for genome maintenance and avoidance of cancer. However, the nature of the S-phase Cdk targets remains largely elusive. We have recently performed a large scale proteomic analysis to identify novel Cdk targets on the chromatin during DNA replication and the DNA damage response. This project will be involved in target validation. The students will clone targets of interest by RT-PCR, express the tagged cDNAs in mammalian cells and analyse chromatin binding using biochemical assays. The project includes a good balance of molecular genetics, biochemical and cell biological techniques and will generate results that are likely to be of general interest.

Life Science Projects 2011-2012

Faculty Name: Dr. Eva Hoffmann
Room No: JMS 2C37/Genome **Email:** eh58@sussex.ac.uk

Project Title/Area: Chromosome segregation at meiosis I

Course requirements: Genetics	**No of places:** 2

Further Information:

Crossing over is important for accurate chromosome segregation at meiosis I. Mis-segregation leads to infertility and aneuploidy (e.g. Down's Syndrome). This project involves identification and characterization of new genes that promote crossing over and chromosome segregation at meiosis I. We use budding yeast as a model organism as fertility studies are possible and easily tractable and since this organism shares many conserved genes with plants and human. The project involves assessing the impact of one or two genes in crossing over and chromosome segregation using genetic, molecular and cytological assays. All these are standard assays in the Hoffmann laboratory. You will be supervised by experts in the various techniques, but will also be given the opportunity to work independently.

Example literature:

Falk *et al*. (2010). "A Mec1- and PP4-dependent checkpoint couples centromere pairing to meiotic recombination". Dev Cell **19**:599-611.

Newnham *et al.* (2010). "The synaptonemal complex protein, Zip1, promotes the segregation of nonexchange chromosomes at meiosis I". PNAS **107**: 781-785.

Life Science Projects 2011-2012

Faculty Name: Prof. Penny Jeggo
Room No: **Email:** p.a.jeggo@sussex.ac.uk

Project Title/Area: DNA damage responses
Examination of DNA double strand break repair in mammalian cells

Course requirements:	**No of places:** 1
Doing 3rd year Genome Damage, genetic disease and cancer	

Further Information:
DNA double strand breaks (DSBs) represent a major lesion that can cause cell death if not repaired or cancer is misrepaired. DSBs are repaired by one of two pathways, DNA non homologous end joining (NHEJ) and homologous recombination. DSBs can now be readily detected in cells by microscopy based procedures exploiting our detailed knowledge of the DNA damage response processes. The procedure of enumerating damage response foci (IRIF) to monitor the process of DSBs repair is being used extensively to gain insight into the mechanisms and interplay between the pathways. The student will participate in our analysis of DSB repair by helping with the enumeration of IRIF in cells of different genetic backgrounds and given different treatments.

Project Title/Area:
Assessment of the genetic susceptibility to low dose radiation exposure

Course requirements:	**No of places:**1
Doing 3rd year Genome Damage, genetic disease and cancer	

Further Information:
X-rays and CT scanning are being increasingly used for medical benefits, resulting in increased exposure of individuals to low doses of radiation. However, whether low dose exposure is harmful and can itself induce cancer is still unclear. Epidemiological studies are insensitive and/or costly to undertake. They need to be coupled with a basic understanding of the biological processes that function in response to low doses of radiatioin. A further important issue is whether there are individuals with genetic predisposition to low doses of radiation. This project will focus on a literature review using pubmed to assess the evidence for genetic susceptibility to low doses of radiation. There will likely also be the possibility to assist with experiments assessing the sensitivity to cells to low dose radiation exposure.

Life Science Projects 2011-2012

<table>
<tr><td colspan="2">Faculty Name: Prof. Alan Lehmann
Room No: G4.08 Email: a.r.lehmann@sussex.ac.uk</td></tr>
<tr><td colspan="2">Project Title/Area: Xeroderma pigmentosum (XP)</td></tr>
<tr><td>Course requirements: Cell Regulation and cancer advisable
3rd year Genome damage, genetic diseases and cancer essential</td><td>No of places: 2</td></tr>
<tr><td colspan="2">Further Information: Students will be required to do a literature review of different aspects of the genetic disorder XP caused by defects in DNA repair. In addition they will have the opportunity to see and speak to patients at the XP multidisciplinary clinic at St Thomas Hospital. From this they will be expected to assess the clinical variability of XP and try and relate it to the molecular defects.

The project is suitable for biomedical scientists. The student must be self-motivated and be able to study independently.</td></tr>
</table>

Life Science Projects 2011-2012

Faculty Name: Dr. Johanne Murray
Room No: G3-04 **Email:** j.m.murray@sussex.ac.uk

Project Title/Area:

Regulation of Recombination

Course requirements:	**No of places:** 2

Further Information:

This project is to use a genetic assay to identify and characterise recombination regulators. Recombination has been called a double edged sword, it is essential to repair some types of DNA damage but it also can lead to genome rearrangements. Chromosome rearrangements are a hall mark of cancer cells and defects in the regulation of recombination, as for example in Bloom's syndrome individuals who carry mutations in the BLM helicase, leads to an increased predisposition to cancer. This shows that the regulation of recombination is important for human health.

The proteins involved in homologous recombination are conserved from yeast to man and mutations in the yeast gene homologous to BLM lead to an increase in genome instability. A good readout of this is in ribosomal DNA, which encodes the ribosomal RNA genes in tandem direct repeats. The copy number of these repeats is species specific and tightly regulated. Copy number is maintained by recombination and so rDNA stability is a good assay for recombination defects. This project will use the fission yeast model system to investigate how recombination in the rDNA is regulated and to identify factors that are important for rDNA stability. This is important for understanding the mechanisms that lead to genome instability and cancer.

The project will involve molecular techniques (PCR, mutagenesis, recombination mediated cassette exchange), yeast genetics and cell biology.

Life Science Projects 2011-2012

Faculty Name: Dr. Matthew Neale
Room No: G4.03 **Email:** m.neale@sussex.ac.uk

Project Title/Area: Investigating DNA repair during meiosis

Course requirements: Biochemistry, Genetics, Molecular Biology or similar	**No of places:** 1

Further Information:

Meiosis is essential for generating genetic variation, thereby influencing evolutionary change. In my lab we investigate the molecular steps of the chromosome transactions that place during meiosis – a process called meiotic recombination – using budding yeast as a simple model system. This is a fascinating process that involves many of the same factors required for cells to resist and repair environmental DNA damage. As such, our studies overlap, involve and inform those studies investigating cell cycle regulation, checkpoint signalling, chromosome biology and classical DNA repair.

Life Science Projects 2011-2012

Faculty Name: Dr. Mark O'Driscoll
Room No: GDSC (G4.19) **Email:**m.o-driscoll@sussex.ac.uk

Project Title/Area:
Literature-based critical review project more suited to the biomed students.
Project 1: The application of genomic technology (array-comparative genomic hybridization, next generation sequencing) to determine the aetiology of Genomic Disease.

Course requirements:	**No of places:** 1

Further Information:

Looking for an in-depth review of the development of a-CGH and next generation technology over the last decade or so and how it is revolutionising disease diagnosis in the clinic. The dissertation will incorporate a description of both techniques as well as reviewing how this technology has shaped our understanding of the underlying mechanisms of genomic structural alterations (deletion, duplication, re-arrangements) causative of these conditions as well as in cancer.

Related information:

DECIPHER,(https://decipher.sanger.ac.uk/), a database collating multiple genomic imbalances and their associated clinical features in human genomic disorders.

EUCARUCA, (http://agserver01.azn.nl:8080/ecaruca/whatisEc.jsp), a database of cytogenetic and clinical data of rare chromosomal aberrations from all centres that are member of the European Cytogeneticists Association (ECA).

Database of Genomic Variants, (http://projects.tcag.ca/variation/), a database listing a comprehensive summary of structural variation in the human genome.

O'Driscoll M. Haploinsufficiency of DNA Damage response genes and their potential influence in human genomic disorders. *Current Genomics 2008 May; 9(3):137-146.*

Pennisi E: Breakthrough of the Year: Human Genetic Variation. *Science* 2007, 318:1842-1843.

Lupski JR: Genomic rearrangements and sporadic disease. *Nat Genet* 2007, 39:S43-47.

Lee C, Iafrate AJ, Brothman AR: Copy number variations and clinical cytogenetic diagnosis of constitutional disorders. *Nat Genet* 2007, 39:S48-54.

Carter NP: Methods and strategies for analyzing copy number variation using DNA microarrays. *Nat Genet* 2007. 39:S1-3.

Sebat J: Major changes in our DNA lead to major changes in our thinking. *Nat Genet* 2007, 39:S3-5.

McCarroll SA, Altshuler DM: Copy-number variation and association studies of human disease. *Nat Genet* 2007, 39:S37-42.

Faculty Name: Dr. Mark O'Driscoll (continued)
Room No: GDSC (G4.19) **Email:**m.o-driscoll@sussex.ac.uk

Project Title/Area:
Literature-based critical review project more suited to the biomed students.

Project 2: The unanticipated consequences of immunosuppressant-based therapies with regard to genomic instability, cancer predisposition and treatment of patients with defects in DNA repair pathways.

Course requirements:	**No of places:** 1

Further Information:

This dissertation will over-view the mechanisms of the most commonly employed immunosuppresants including cyclosporin, rapamycin, azathioprine and mycophenotale mofetil, describing how each targets different signalling systems. The undesirable outcomes of immunosuppression will then be outlined contrasting loss of host immune-surveillance mechanisms with recently described overt genotoxicity of agents such as cyclosporin and azathioprone. With respect to the latter, a detailed review of the models of its genotoxicity will be presented. Furthermore, a detailed description of how this genotoxicity affects the treatment options available to individuals suffering from congenital defects in the DNA repair pathways (e.g LIG4 syndrome, Nijmegen breakage syndrome, Fanconi anaemia).

O'Driscoll M and Jeggo P.A Cyclosporine A can induce DNA double strand breaks: implications for bone marrow transplantation regimens particularly for individuals with defective DNA repair. *Bone Marrow Transplantation 2008 Jun 1;41(11):983-989.*

Aschan J. Allogeneic haematopoietic stem cell transplantation: current status and future outlook. *Br Med Bull. 2006 January 1, 2006;77-78(1):23-36.*

O'Marcaigh A, DeSantes K, Hu D, Pabst H, Horn B, Li L, et al. Bone marrow transplantation for T-B- severe combined immunodeficiency disease in Athabascan-speaking native Americans. *Bone Marrow Transplantation. 2001;27(7):703-9.*

Buckley RH. Molecular Defects in Human Severe Combined Immunodeficiency and Approaches to Immune Reconstitution. *Annual Review of Immunology. 2004;22(1):625-55.*

Ruutu T, Niederwieser D, Gratwohl A, Apperley J. A survey of the prophylaxis and treatment of acute GVHD in Europe: a report of the European Group for Blood and Marrow Transplantation (EBMT). *Bone Marrow Transplantation. 1997;19(8):759-64.*

Deeg HJ, Socie G. Malignancies After Hematopoietic Stem Cell Transplantation: Many Questions, Some Answers. *Blood. 1998;91(6):1833-44.*

Buell JF, Gross TG, Woodle, E Steve Malignancy after transplantation. *Transplantation. 2005;80(2(S)):254-64.*

O'Donovan P, Perrett CM, Zhang X, Montaner B, Xu Y-Z, Harwood CA, et al. Azathioprine and UVA Light Generate Mutagenic Oxidative DNA Damage. *Science. 2005;309(5742):1871-4.*

Karran P. Thiopurines, DNA damage, DNA repair and therapy-related cancer. *Br Med Bull. 2006;79-80(1):153-70.*

Life Science Projects 2011-2012

Faculty Name: Dr. Antony W. Oliver
Room No: G4.02 (Genome) **Email:** antony.oliver@sussex.ac.uk

Project Title/Area:
Laboratory Based Project — Characterisation of human TopBP1 and / or Schizosaccharomyces pombe Rad4 phospho-peptide interactions**.**

Course requirements:	**No of places:** 1 (one)
2nd Year Module - Proteins in Action	

Further Information:

Briefly, the project will involve — expressing in and purifying from E.coli, different expression constructs of TopBP1 and Rad4; site-directed mutagenesis of key amino-acid residues involved in phospho-peptide binding; characterisation of phospho-peptide binding by Fluorescence Polarisation.

1. Rappas, M., Oliver, A.W. and Pearl L.H. (2011). "Structure and function of the Rad9-binding region of the DNA-damage checkpoint adaptor TopBP1". Nucleic Acids Res. 2011 Jan 1;39(1):313-24.

2. Garcia V., Furuya K., Carr A.M. (2005). "Identification and functional analysis of TopBP1 and its homologs". DNA Repair (Amst). 2005 Nov 21;4(11):1227-39.

Project Title/Area:
Critical review — The different roles of the poly-BRCT protein TopBP1 in DNA replication and DNA damage repair.

Course requirements:	**No of places:** 1 (one)

Further Information:

The project will involve a critical review, incorporating existing literature, around the differing roles of the poly-BRCT protein TopBP1 in the processes of DNA replication and DNA damage repair, and the relevance of mutations of TopBP1 in human disease.

1. Rappas, M., Oliver, A.W. and Pearl L.H. (2011). "Structure and function of the Rad9-binding region of the DNA-damage checkpoint adaptor TopBP1". Nucleic Acids Res. 2011 Jan 1;39(1):313-24.

2. Garcia V., Furuya K., Carr A.M. (2005). "Identification and functional analysis of TopBP1 and its homologs". DNA Repair (Amst). 2005 Nov 21;4(11):1227-39.

Life Science Projects 2011-2012

Faculty Name: Dr. Hideo Tsubouchi
Room No: JMS2C34 **Email:** h.tsubouchi@sussex.ac.uk

Project Title/Area:
Characterization of human Rad51AP2, a meiosis-specific protein that interacts with homologous recombinase Rad51

Course requirements: none	**No of places:** 1

Further Information:
Homologous recombination is essential for accurate segregation of chromosomes in meiosis. Missegregation of chromosomes in meiosis leads to natural abortion and various birth defects including Down's syndrome caused by the acquisition of an extra copy of chromosome 21. Rad51 is a eukaryotic homolog of bacterial homologous recombinase RecA, playing a central role in homologous recombination. To further understand the mechanism to regulate homologous recombination, we previously searched for proteins that interact with Rad51, and isolated a protein called Rad51AP2 as a candidate. To further characterize this protein, the following experiments are being planned. First, to investigate the localization of Rad51AP2 by indirect immunostaining, antibodies against Rad51AP2 will be raised. For this purpose part of Rad51AP2 will be produced in bacteria and purified as an antigen. Second, to characterize the biochemical properties of Rad51AP2, the full length of the Rad51AP2 will be purified. To directly assess the physical interaction between Rad51 and Rad51AP2, Rad51 will be purified as well.

Project Title/Area:
Establishing a cell biology-based method for measuring meiotic recombination using budding yeast

Course requirements: none	**No of places:** 1

Further Information:
Homologous recombination is essential for accurate segregation of chromosomes in meiosis. Missegregation of chromosomes in meiosis leads to natural abortion and various birth defects including Down's syndrome caused by the acquisition of an extra copy of chromosome 21. At the first division of meiosis (meiosis I), homologous chromosomes are segregated, which is distinct from mitosis where sister-chromatids are segregated. For homologs to be accurately segregated, homologs need to recognize each other and then physical links need to be established between them. Homologous recombination plays essential roles in these processes. The physical links between homologous chromosomes are mediated by reciprocally exchanged DNA strands, or crossovers. The distribution and number of crossovers are precisely controlled but the underlying mechanism is not clear partly because of the lack of robust system to detect multiple crossovers in a cell. In this project, by using three color fluorescent proteins (variants of Green Fluorescent Protein: BFP, YFP, RFP), we will try to make a new microscope-based system to detect crossovers with budding yeast meiosis.

Life Science Projects 2011-2012

Faculty Name: Dr. Felicity Watts
Room No: G4.12 **Email:** f.z.watts@sussex.ac.uk

Project Title/Area:
Characterisation of the BRCT domains of the BRCA1-related protein Crb2 (lab based)

Course requirements: Molecular Genetics would be useful	**No of places:** 2

Further Information:

DNA integrity checkpoints ensure that cells do not enter mitosis in the presence of DNA damage. They are essential if cells are to maintain genetic integrity and hence prevent events such as chromosome loss and cancer. Checkpoints comprise DNA damage sensors, mediators and signal transducers, and are generally conserved in eukaryotes. Crb2 acts as a mediator protein in *S. pombe*.

Crb2 is closely related to the mammalian DNA integrity checkpoint protein 53BP1 (p53-binding protein). It is also related to BRCA1, mutations in which are responsible for a high proportion of familial breast and ovarian cancers. Crb2, 53BP1 and BRCA1 all contain BRCT domains at their C-termini. Many of the cancer-causing mutations in BRCA1 map to the BRCT domains, testifying to their importance in maintainence in genetic integrity. By combining biophysical, biochemical and genetic techniques, we have initiated a structure/function analysis of the BRCT domains in Crb2. From the 3-D structure of the BRCT domains determined by X-ray crystallography, we have identified amino acids that are required either for dimerisation of Crb2 or separately, that are required to bind phosphopeptides (Kilkenney *et al*., 2008). One of the phosphoproteins with which Crb2 interacts is phosphorylated histone H2A (γ-H2A). Our genetic studies suggest that Crb2 likely also interacts with other phosphoproteins. Taking a bioinformatic approach we have identified a number of proteins that are candidates for interaction with Crb2. The aim of this study is to test whether any of these proteins actually interact with Crb2. The project will involve a combination of genetic and biochemical approaches. Specifically, the student will use site-directed mutagenesis on genes encoding candidate proteins. Mutant sequences will be introduced into cells and the phenotypes observed and compared with that of a crb2 mutant in which phosphoprotein binding no longer occurs. Double mutants will also be analysed to determine whether the mutations affect the same or different pathways. The interaction will also be tested biochemically using recombinant forms of the proteins. Information gained from such a study will be applicable to the understanding of checkpoint function in mammalian cells.

Techniques: Site-directed mutagenesis, sequencing, yeast transformation, genetics, analysis of DNA damage responses

Kilkenney, M.L. Dore, A., Roe, S.M., Nestoras, K., Ho, J.C.Y., Watts, F.Z. and Pearl, L.H. Structural and functional analysis of the Crb2-$BRCT_2$ domain reveals distinct roles in checkpoint signalling and DNA damage repair Genes and Dev. (2008) 22, 2034-47.

Faculty Name: Dr. Felicity Watts (continued)
Room No: G4.12 **Email:** f.z.watts@sussex.ac.uk

Project Title/Area:

Analysis of BRCA1 mutations (non lab based)

Course requirements:	**No of places:** 1

Further Information:

DNA integrity checkpoints ensure that cells do not enter mitosis in the presence of DNA damage. They are essential if cells are to maintain genetic integrity and hence prevent events such as chromosome loss and cancer. Checkpoints comprise DNA damage sensors, mediators and signal transducers, and are generally conserved in eukaryotes. Crb2 acts as a mediator protein in *S. pombe*.

Crb2 is closely related to the mammalian DNA integrity checkpoint protein 53BP1 (p53-binding protein). It is also related to BRCA1, mutations in which are responsible for a high proportion of familial breast and ovarian cancers. Crb2, 53BP1 and BRCA1 all contain BRCT domains at their C-termini. Many of the cancer-causing mutations in BRCA1 map to the BRCT domains, testifying to their importance in maintenance in genetic integrity. We have recently determined the structure of the Crb2 BRCT domains and are interested in their role.

The aim of this project is to do a survey of the published literature on the identified mutations in the BRCA1 gene. Having done this the position of the mutations within the different domains should be analysed, in particular mutations mapping to the BRCT domains. Using sequence analysis programs the BRCT domains of Crb2 and BRCA1 will next be compared and the positions of the BRCA1 mutations noted. Following this molecular modelling will be used to map the sites of the BRCA1 mutations on the known crystal structure.

Project Title/Area:
Analysis of the role of SUMO (small ubiquitin-like modifier) in the DNA damage response (lab based)

Course requirements:	**No of places:** 1

Further Information:

SUMO is a small ubiquitin-like modifier. Using fission yeast as a model system, we have shown that SUMO-modification of proteins is required for the DNA damage response. To identify SUMO modified proteins we have tested a number of known DNA damage response proteins for their ability to be modified *in vitro* and *in vivo*. So far we have identified a number of proteins required for DNA replication and recombination. The aim of this project is to take a more global approach. We have established conditions for 2D gel analysis of yeast proteins. In addition we have two sumoylation mutants, one which results in reduced SUMO chain formation and another which results in stabilisation of SUMO-modified targets. Specifically, this project will involve a comparison of SUMO-modified species in the two different mutant strains with those observed in wild type cells. Cell will also be treated with DNA damaging agents to determine whether the pattern of modified species alters under different conditions.