Life Science Project Titles 2012-2013

Genome, Damage and Stability Subject Area

Life Science Projects 2012-2013

Faculty Name: Dr Jon Baxter

Room No: G4:15 **Email**:Jon.Baxter@sussex.ac.uk

Project Title/Area:

Derivation of novel condensin mutants for the analysis chromosome resolution

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

Further Information:

This project will combine yeast genetics and molecular biology to derive novel “degron” mutants of the yeast condensin genes.

Life Science Projects 2012-2013

Faculty Name: Dr Alessandro Bianchi

Room No: JMS 2C37 **Email:** a.bianchi@sussex.ac.uk

Project Title/Area:

Investigation of the DNA binding properties of the telomere protein Rif1

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

Further Information:

The Rif1 (Rap1 Interacting Factor 1) gene was initially identified in budding yeast as a factor capable of interacting with a major telomere-binding protein, Rap1, which binds to double-stranded (DS) telomeric DNA repeats hereby recruiting Rif1 to telomeres. Yeast Rif1 has since been shown to be a key regulator of telomerase activity at telomeres and to also be a player in the initiation of DNA replication. More recent work has uncovered a role for Rif1 in suppressing the DNA damage checkpoint at telomeres, in a manner that might be independent of Rap1. In particular, it has been proposed that Rif1 might compete proteins in the DNA damage/DNA repair pathway for binding to damaged DNA. Proposed DNA substrates for Rif1 binding include single-stranded (SS) DNA and the junction between SS and DS DNA that is created during the processing of damaged DNA. In order to test the idea that Rif1 might be capable of binding DNA directly, we have generated constructs for the expression of Rif1 protein in insect cells. The project will involve the culturing of these cells, their infection with the insect-specific virus for expressing Rif1, the preparation of purified Rif1 protein using standard affinity purification methods, and the assaying of the putative DNA binding activity of Rif1 using fluorescent-labelled DNA substrates in electrophoresis-mobility-shift-assays (EMSAs).

Life Science Projects 2012-2013

Faculty Name: Dr. Alessandro Bianchi

Room No: JMS 2C37 **Email:** a.bianchi@sussex.ac.uk

Project Title/Area:

Isolation of fission yeast mutants defective in protein-protein interactions at the telomeric complex using a reverse two-hybrid approach

Course requirements:	**No of places:** 1 or 2

Further Information:

The fission yeast telomeric complex has a very high degree of homology to the human complex and therefore it constitutes an excellent system for functional studies. Most of the key telomeric protein components are conserved between fission yeast and humans and so are several of the interactions between these components. With regard to telomerase activation the protein factors of the complex can be broadly divided into two categories: those that bind (directly or indirectly) to the internal double-stranded (DS) telomeric DNA repeats, and those that bind (directly or indirectly) to the single-stranded (SS) terminal DNA overhangs. Whereas the first class of factors inhibit telomerase activity, the second class promote it (and are actually required for it). Interestingly, the two classes of factors are connected by protein-protein interactions and several factors in the complex have dual, bridging, interactions. A model for telomerase regulation in yeast and humans proposes that the SS-DNA binding complex can be sequestered in an inactive form by the DS DNA binders, in a manner that is dependent on telomere length. In this manner longer telomeres would be prevented by becoming over-elongated. This model predicts that mutations within several factors that would disrupt interactions between the two groups of factors would lead to telomere elongation. We will test this model by generating alleles of key factors that lack the ability to bind either the inhibitory or the activating group of proteins. To this goal we will use a two-hybrid approach, a classic method to genetically score physical interactions between protein factors in vivo. In particular we will use a variant of this method specifically designed to isolate mutations affecting to the interaction of a protein for one, but not the other, of its partners. The project will involved standard molecular genetic techniques in yeast.

Life Science Projects 2012-2013

Faculty Name: Professor Keith Caldecott

Room No: **Email**:k.w.caldecott@sussex.ac.uk

Project Title/Area:

Expression, purification, and (depending on progress), characterisation of DNA repair proteins implicated in the maintenance of genetic stability

Course requirements:	**No of places**: 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. If progress allows, some cellular analyses of protein function and genetic stability will be conducted.

Life Science Projects 2012-2013

Faculty Name: Professor Keith Caldecott

Room No: **Email:** k.w.caldecott@sussex.ac.uk

Project Title/Area:

Link between mutations in DNA single-strand break repair/base excision repair genes and cancer incidence (dry project)

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

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.

Life Science Projects 2012-2013

Faculty Name: Professor Antony Carr

Room No: G4-18 **Email**: a.m.carr@sussex.ac.uk

Project Title/Area:

Function of Exo1 (nuclease) in maintaining genome stability.

Course requirements:

Biology/Biochemistry

No of places: 2

Further Information:

ExoI is a nuclease that is regulated in response to replication stress by the DNA replication checkpoint (Reference Genes Dev. 2008 Jul 1;22(13):1816-27. Separate roles for the DNA damage checkpoint protein kinases in stabilizing DNA replication forks: Segurado M, Diffley JF). The regulation of DNA metabolism at stressed replication forks is a fundamental process that protects us from the genomic rearrangements that are precursors to cancer. We are interested to know how DNA metabolism is orchestrated at stressed replication forks in the model organism S. pombe (fission yeast) and how this regulation impacts on genome stability and cell survival.

Towards this understanding, the project will create and/or use plasmid reagents and engineered yeast strains to makes site specific mutations in a specific DNA repair gene (*exo1*) 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.

Life Science Projects 2012-2013

Faculty Name: Professor Aidan Doherty

Room No: G3-09 **Email** : ajd21@sussex.ac.uk

Project Title/Area:

Mechanisms of DNA double-strand break repair by a non homologous end-joining complex

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

Further Information:

DNA double-strand breaks (DSBs) are one of the most lethal forms of DNA damage, as even a single DSB is sufficient to kill a cell. Incorrectly repaired, or unrepaired breaks can lead to gross chromosomal rearrangements, aneuploidy and ultimately, carcinogenesis and cell-death.

Non homologous end-joining (NHEJ) is a major DNA double-strand break repair pathway (DSB) in both prokaryotes and eukaryotes. A core protein complex comprising Ku and DNA ligase assembles at DSBs to mediate repair of broken DNA ends.

The project will involve the use of a variety of biochemical and molecular biology techniques (e.g. cloning, protein purification and DNA repair assays) to elucidate the mechanism of action of the bacterial NHEJ repair complex.

References

1. Brissett (2011) Mol. Cell 41, 221-231.

2. Brissett et al (2007) Science 318, 456-459.

3. Bowater & Doherty (2006) PLOS Genetics 2, e8.

Life Science Projects 2012-2013

Faculty Name: Professor Aidan Doherty

Room No: G3-09 **Email:** ajd21@sussex.ac.uk

Project Title/Area:

Molecular role of novel eukaryotic proteins in DNA repair

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

Further Information:

Recent work from my laboratory has implicated a novel family of proteins in the repair of DNA in eukaryotes, including humans. The aim of this project is to elucidate the structure and function of these novel enzymes. This research will delineate the molecular role played by these enzymes in repairing DNA in eukaryotic cells. The outcome will directly impact on our understanding of the cellular mechanisms that propagate and repair DNA and will inform the development of diagnostic tools to identify mutations associated with human diseases and develop novel inhibitors that can treat disease and infection.

The project will involve the use of a variety of biochemical and molecular biology techniques (e.g. cloning, protein purification and DNA repair assays) to elucidate the mechanism of action of the bacterial NHEJ repair complex.

Reference

Eukaryotic translesion polymerases and their roles and regulation in DNA damage tolerance.

Waters LS, Minesinger BK, Wiltrout ME, D'Souza S, Woodruff RV, Walker GC.

Microbiol Mol Biol Rev. (2009) 73, 134-54.

Life Science Projects 2012-2013

Faculty Name: Professor Aidan Doherty

Room No: G3-09 **Email:** ajd21@sussex.ac.uk

Project Title/Area:

Regulation of NHEJ repair pathway in eukaryotes

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

Further Information:

DNA double-strand breaks (DSBs) are one of the most lethal forms of DNA damage, as even a single DSB is sufficient to kill a cell. Incorrectly repaired, or unrepaired breaks can lead to gross chromosomal rearrangements, aneuploidy and ultimately, carcinogenesis and cell-death. Non homologous end-joining (NHEJ) is a major DNA double-strand break repair pathway (DSB) in both prokaryotes and eukaryotes.

The project will study the NHEJ pathway in the model eukaryotic organism, S.pombe and how it is regulated during G1 phase of the cell cycle. The project will involve the use of a variety of biochemical , genetic and molecular biology techniques

Reference

Hentges, P., Ahnesorg, P., Pitcher, R.S., Bruce, C.K., Kysela, B., Green, A.J., Bianchi, J. Wilson, T.E., Jackson S.P. & **Doherty, A.J.**

Evolutionary and Functional Conservation of the DNA Non homologous End-joining Protein, XLF/Cernunnos.

J. Biol. Chem. 281, 37517–37526.

Life Science Projects 2012-2013

Faculty Name: Dr Jessica Downs

Room No: G4.12 **Email**: j.a.downs@sussex.ac.uk

Project Title/Area:

Analysis of DNA damage induced phosphorylation of the chromatin remodelling complex subunit Rsc2

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

Further Information:

The Rsc2 subunit of the RSC chromatin remodelling complex plays an important role in preventing genome instability. It has recently been found to be phosphorylated in response to DNA damage. In this lab based project, the student will investigate the functional significance of this phosphorylation to determine how this helps Rsc2 maintain genome stability. The project will involve cloning and cell based survival assays using budding yeast as a model system. The data generated during the project can be related to the human homologue of Rsc2, termed BAF180, which is mutated in over 40% of renal cell carcinomas.

Life Science Projects 2012-2013

Faculty Name: Dr Jessica Downs

Room No: G4.12 **Email:** j.a.downs@sussex.ac.uk

Project Title/Area:

Analysis of the chromatin remodelling subunit BAF180 in cancer

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

Further Information:

The gene encoding BAF180 (PBRM1) is frequently mutated in cancer. In this literature-based project, the student will undertake a systematic analysis of the mutational spectrum of the gene and correlate this with tumour type. This will involve a comprehensive literature review combined with analysis of existing cancer databases. The relevance of PBRM1 mutation, and consequently BAF180 function, to cancer on a molecular level will also be examined.

Life Science Projects 2012-2013

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 2012-2013

Faculty Name: Dr. Helfrid Hochegger

Room No: G3.09 **Email**: hh65@sussex.ac.uk

Project Title/Area:

Generating gene targeting constructs to knock out vertebrate Katanin

Course requirements:	**No of places**: 1
Good Grades and Enthusiasm	

Further Information:

Katanin's are microtubule-severing enzymes that are important for mitosis. Their precise function remains unknown. We are planning to generate Katanin knock out cells to study the function of these interesting enzymes. In this project, the student will learn molecular biology techniques such as PCR, subcloning, recombeneering and in the later stages cell biology and gene targeting techniques.

Life Science Projects 2012-2013

Faculty Name: Dr. Eva Hoffmann

Room No: Genome 2C37 **Email**: eh58@sussex.ac.uk

Project Title/Area:

Identification of new mismatch repair genes and implication for hereditary non-polyposis colorectal cancer

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

Further Information:

We have identified two new mutator genes that may be implicated in mismatch repair, important for the correction of mis-incorporated dNTPs in the newly-synthesized strand of the DNA helix. Further characterization is needed to understand whether these two candidate genes are directly involved in mismatch repair or whether they become important for mutation avoidance when mismatch repair is compromised. To investigate these potential link, mutation rate assays will be carried out on various mutants, in combination with deletions of mismatch repair genes. Nuclear extract from these mutations will be investigated for their ability of repair defined mismatches incorporated in plasmid and/or oligonucleotide DNA. This work may lead to the identification of new genes associated with the HNPCC syndrome.

Life Science Projects 2012-2013

Faculty Name: Dr. Eva Hoffmann

Room No: Genome 2C37 **Email:** eh58@sussex.ac.uk

Project Title/Area:

Bioinformatic and literature review of mutator genes and possible implication in cancer

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

Further Information:

We have completed a genome-wide screen of 4,700 mutants for change mutation rates when mismatch repair is compromised. This project will take the results and analysis the hits (~ 1200) into gene ontology categories as well as do a critical literature survey to establish whether specific clusters may be implicated in cancer.

Life Science Projects 2012-2013

Faculty Name: Professor Alan Lehmann

Room No: G4.08 **Email**: a.r.lehmann@sussex.ac.uk

Project Title/Area:

Xeroderma pigmentosum (XP)

Course requirements

Cell Regulation and cancer advisable

3rd year Genome damage, genetic diseases and cancer essential

No of places: 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 and clinicians 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.

Life Science Projects 2012-2013

Faculty Name: Dr. Jo 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 2012-2013

Faculty Name: Dr. Jo Murray

Room No: G3-04 **Email:** j.m.murray@sussex.ac.uk

Project Title/Area:

Literature review

Smc5/6 and genome variation

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

Further Information:

The essential and highly conserved Smc5/6 complex is involved in higher order chromosome structure, chromosome segregation and the regulation of homologous recombination (Murray and Carr, 2008). Recombination regulators such as BLM, defective in Bloom's syndrome, are important for genome stability and loss of BLM leads to increased predisposition to all types of cancer. This project is to investigate the in silico data which could suggest that misregulation of Smc5/6 components might be associated with cancer predisposition.

Life Science Projects 2012-2013

Faculty Name: Dr. Matt Neale

Room No: G4.13 /G4.12 **Email:** m.neale@sussex.ac.uk

Project Title/Area:

Optimising genetic modification (transformation) of Saccharomyces cerevisie

Course requirements:	**No of places**: 2
Molecular Biology, Genetics	

Further Information:

The ability to genetically manipulate and modifiy chromosomal sequences (transformation) is an essential tool of modern molecular biology and genetics. However, the efficiency of generating stable chromosomal changes is very low, hampering scientific research. These two projects will investigate and optimise the protocols used to create genomic modifications. The study will utilise the buding yeast Saccharomyces cerevisiae as the model system to investigate this process. The project will involve yeast cell culturing, DNA preparation and manipulation. The project will suit molecular biology and genetics students who enjoy problem solving and who wish to get additional experience in laboratory research.

Life Science Projects 2012-2013

Faculty Name: Dr. Mark O'Driscoll

Room No: G4.03 **Email**: m.o-driscoll@sussex.ac.uk

Project Title/Area:

Understanding the adipo- and lipogenic impacts of impaired ATR function.

Laboratory-based project.

Course requirements:	**No of places**: 1
Biochem, cell biology	

Further Information:

ATR is a central controller of the DNA damage response, a network governing cell cycle checkpoint arrest, DNA repair and apoptosis. Congenital defects in ATR-pathway cause Seckel syndrome, a severe primordial microcephalic dwarfism. Inhibitors of ATR are currently being pursued as a novel cancer chemotherapy. Our data suggests an adverse impact upon normal metabolism when ATR is limiting. This project will involve examining the impact of chemical inhibition of ATR on adipogenesis (fat cell formation) and liogenesis (fat production) using a model tissue culture system. This is of relevance to insulin resistance and cancer-associated cachexia. Depending on these findings, the candidate will engineer mutations by site-directed mutagenesis in the LIP-I lipase to examine the functional role of a proposed ATR consensus phosphorylation site upon LIP-I activity.

This is a laboratory-based project involving tissue culture, drug handling and molecular biology-based techniques.

Life Science Projects 2012-2013

Faculty Name: Dr. Mark O'Driscoll

Room No: G4.03 **Email:** m.o-driscoll@sussex.ac.uk

Project Title/Area:

Overview of the current situation regarding the potential use of small molecule inhibitors of DNA repair

Literature-based project

Course requirements:	**No of places: 1**
Suited for intercalating med student	

Further Information:

Several small molecule inhibitors of important players in the DNA repair network have progressed from the lab into clinical trials. There has been much exciting in their application as synthetically lethal tools that exploit underlying deficits of certain cancer types. This literature-based review will entail **1).** describing the mechanisms of action of chemical inhibitors of ATM, ATR, DNA-PKcs and PARP, **2).** of detailing the context proposed for their clinical employment and **3).** overviewing the latest published information concerning clinical trials of these inhibitors where available.

Life Science Projects 2012-2013

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.

Life Science Projects 2012-2013

Faculty Name: Dr. Antony W. Oliver

Room No: G4.02 (Genome) **Email:** antony.oliver@sussex.ac.uk

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**

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 2012-2013

Faculty Name: Dr. Steve Sweet

Room No: G3.05 **Email**: s.m.sweet@sussex.ac.uk

Project Title/Area:

Histone modifications and DNA damage repair

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

Further Information:

The DNA damage response (DDR) occurs in the background of chromatin.[1,2] A number of histone PTMs and variants are known to be involved in the DDR. The DDR initially entails histone H4 acetylation and opening up of chromatin structure. Kinases are recruited, resulting in extensive H2A.X phosphorylation. DNA is resected and nucleosomes removed around the site of repair. Subsequent to repair, histone deacetylases are recruited, H2A.X is dephosphorylated and nucleosomes are reincorporated. New H3.1 is incorporated in a CAF-dependent manner.[3]

In this project the student will further develop an *S. pombe* plasmid system[4], allowing targeted and inducible DNA double-strand breaks and affinity purification for subsequent analysis.

Techniques used: molecular biology cloning techniques; yeast culture; affinity purification; SDS-PAGE protein gel electrophoresis.

References:

1. van Attikum, H. & Gasser, S.M. The histone code at DNA breaks: a guide to repair? Nat Rev Mol Cell Biol 6, 757-765 (2005).
2. Luijsterburg, M.S. & van Attikum, H. Chromatin and the DNA damage response: The cancer connection. Molecular Oncology 5, 349-367 (2011).
3. Polo, S.E., Roche, D. & Almouzni, G. New Histone Incorporation Marks Sites of UV Repair in Human Cells. Cell 127, 481-493 (2006).
4. Watson, A.T., Werler, P. & Carr, A.M. Regulation of gene expression at the fission yeast Schizosaccharomyces pombe urg1 locus. Gene 484, 75-85 (2011)

Life Science Projects 2012-2013

Faculty Name: Dr. Hideo Tsubouchi

Room No: JMS 2C34 **Email**: h.tsubouchi@sussex.ac.uk

Project Title/Area:

Molecular mechanism for the inhibition of Rad51, a major eukaryotic homologous recombinase, by Hed1, a meiosis-specific protein.

Course requirements: none

No of places: 2

Further Information:

Homologous recombination is a highly conserved mechanism, conserved from virus to humans. It is crucial for maintaining genome integrity and accurately repairing DNA damage. Homologous recombination is also essential for chromosome segregation during meiosis. Rad51 is a eukaryotic homolog of bacterial RecA protein, playing the central role in homologous recombination.

Previously we found that Hed1, a meiosis-specific protein, strongly inhibits the function of Rad51 during meiosis. To further understand the molecular mechanism of the Rad51 inhibition by Hed1, we aim to further determine how these proteins recognize each other.

We use a system called the yeast two hybrid analysis to systematically characterize protein-protein interactions. The aims are the followings:

(1) To identify the minimum region of Hed1 to support the Hed1-Rad51 interaction

(2) To identify the minimum region of Rad51 to support the Hed1-Rad51 interaction

(3) To examine if the minimum regions isolated above is sufficient to support the Hed1-Rad51 interaction *in vivo*.

The project potentially provides opportunities for students to get familiarized with basic genetics, molecular biology, biochemistry and cell biology.

Busygina, V., Saro, D., Williams, G., Leung, W.K., Say, A.F., Sehorn, M.G., Sung, P., and Tsubouchi, H. (2011). Novel attributes of Hed1 affect dynamics and activity of the Rad51 presynaptic filament during meiotic recombination. J Biol Chem

Busygina, V., Sehorn, M.G., Shi, I.Y., Tsubouchi, H., Roeder, G.S., and Sung, P. (2008). Hed1 regulates Rad51-mediated recombination via a novel mechanism. Genes Dev *22*, 786-795.

Tsubouchi, H., and Roeder, G.S. (2006). Budding yeast Hed1 down-regulates the mitotic recombination machinery when meiotic recombination is impaired. Genes Dev *20*, 1766-775.

Life Science Projects 2012-2013

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

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.

Life Science Projects 2012-2013

Faculty Name: Dr. Felicity Watts

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**
Molecular genetics would be useful	

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.

Life Science Projects 2012-2013

Faculty Name: Dr. Felicity Watts

Room No: G4.12 **Email:** f.z.watts@sussex.ac.uk

Project Title/Area:

Analysis of the role of SUMO (small ubiquitin-like modifier) in the function of Sla1 (homologue of human La protein) (lab-based)

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

Further Information:

SUMO is a small ubiquitin-like modifier, that can be covalently attached to target proteins. Sumoylation is required for a range of biological functions including regulation of transcription, DNA repair, cell cycle and RNA stability. At the molecular level, SUMO modification acts to alter protein-protein interactions, protein-DNA interactions, enzyme activity and protein localisation.

The mammalian La protein is an RNA binding protein and is one of two clinically important autoantigens that are key translational biomarkers for lupus and primary Sjögren's syndrome. Autoantibodies against the La protein mediate tissue damage in the neonatal lupus syndrome, a model of passively acquired autoimmunity in humans in which the most serious manifestation is congenital heart block (CHB).

Using fission yeast as a model system, we have shown that the homologue of the La protein is modified by SUMO on two specific lysine residues. The aim of this project is to identify the role of this sumoylation. Specifically, the project will involve site-directed mutagenesis of the sla1 sequence to create 2 lysine to arginine mutations, to introduce these sequences into fission yeast and to determine the effect of these mutations on cell viability and response to a range of different stresses.

Life Science Projects 2012-2013

Faculty Name: Dr. Felicity Watts

Room No: G4.12 **Email:** f.z.watts@sussex.ac.uk

Project Title/Area:

Analysis of mutations in translation factors in cancer patients (non-lab based)

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

Further Information:

Tumorigenesis and invasive cancer can occur through the disruption of a number of different cellular processes, which includes DNA damage repair and protein synthesis. It is hypothesised that a highly regulated translational apparatus allows a cell to couple rates of protein synthesis to their rates of proliferation. However, when protein synthesis is over-activated, "weak" mRNAs are translated relatively more efficiently, leading to an imbalance of proteins made. Such "weak" mRNAs encode numerous proteins involved in promoting cell growth and proliferation; when such protein levels increase due to the dysregulation of overall protein synthesis, cells become malignant

The aim of this project is to survey the published literature and databases of cancer-associated mutations, to identify translation initiation or elongation factors that show altered expression or which are mutated in cancer cells. Having identified such translation factors, molecular modelling will be used to map the mutations onto the structures of the proteins, where crystal structures are available.

Life Science Projects 2012-2013

Faculty Name: Dr. Felicity Watts

Room No: G4.12 **Email:** f.z.watts@sussex.ac.uk

Project Title/Area:

Analysis of mutations in sumoylation factors associated with human diseases (non-lab based)

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

Further Information:

SUMO is a small ubiquitin-like modifier, that can be covalently attached to target proteins. Sumoylation is required for a range of biological functions including regulation of transcription, DNA repair, cell cycle and RNA stability. At the molecular level, SUMO modification acts to alter protein-protein interactions, protein-DNA interactions, enzyme activity and protein localisation. In order to be attached to target proteins, precursor SUMO is first processed to the mature form. It is then activated by a SUMO activating enzyme and then with the aid of a SUMO conjugating enzyme and in some cases, a SUMO ligase it is attached to target proteins. Mutations is these enzymes have begun to be identified in patients with a range of different diseases

The aim of this project is to survey the published literature and databases to compile a list of diseases caused by mutations in sumoylation factors, and to identify the different mutations. . Molecular modelling will then be used to map the mutations onto the structures of the enzymes, for which crystal structures are already available.