PHENOTYPE

Issue 8, Hilary Term 2011

Research Highlights

FTO SNPs and obesity
DNA repair in yeast
New DNA findings from Oxford

Tropical Ghosts in Oxford

An interview with the artist behind the *Ghost Forest* exhibition

Hypoxia and DNA Damage

Dr Ester Hammond discusses new treatments for hypoxic tumours

5' with...

Professor Judy Armitage

Quorum Sensing

It's all talk between bacteria

Clinical Trials

The career path for you?

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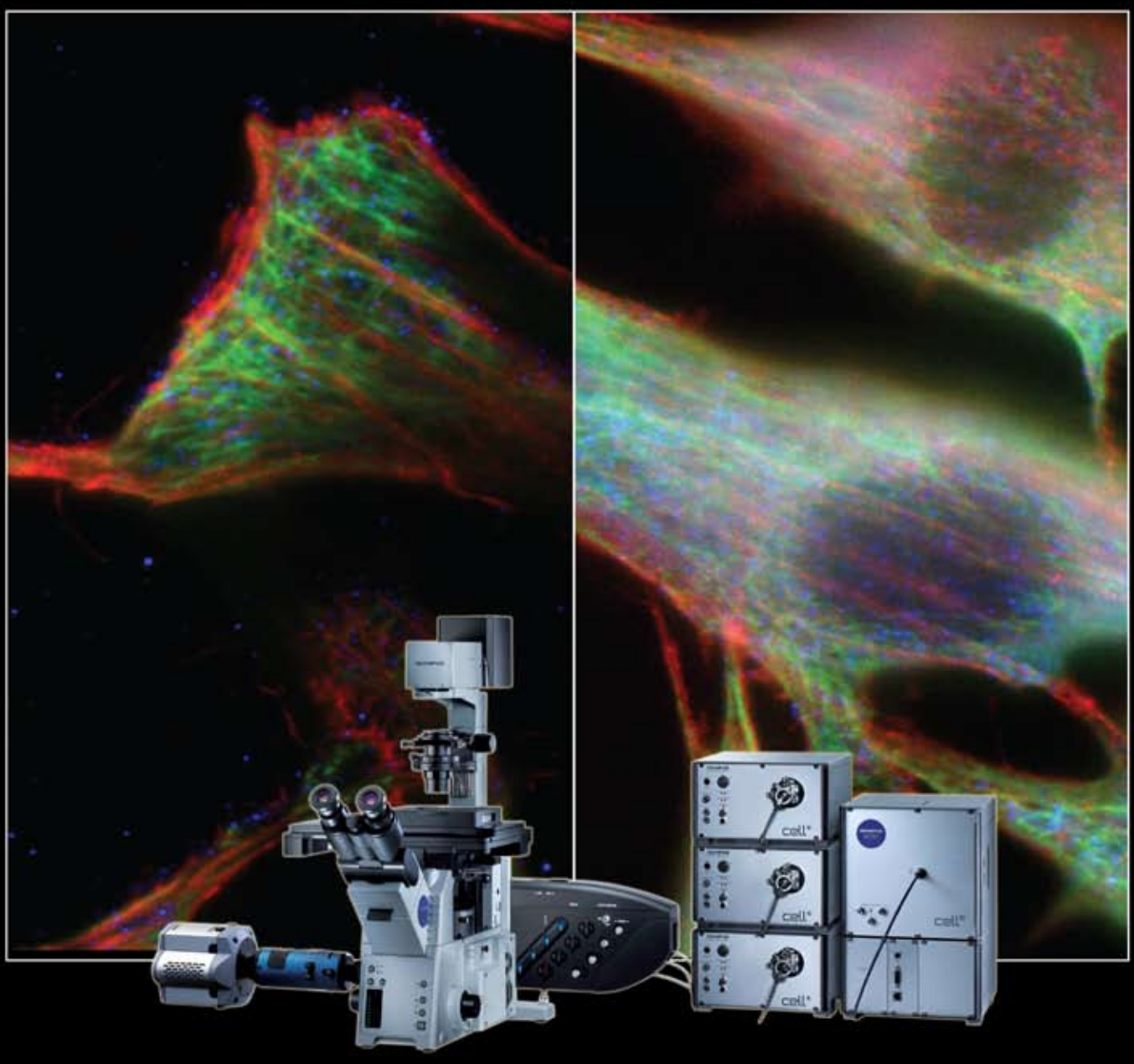

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EDITORIAL

Welcome back to Oxford! I hope you all had a great vacation, and found time to enjoy the rare delights of a white Christmas.

Our Hilary 2011 issue is featuring many exciting articles on life-science related topics, ranging from epigenetics to science in art.

One of the highlights of this issue is the contribution by Dr Ester Hammond, a Cancer Research UK Junior Group Leader at the Gray Institute for Radiation Oncology and Biology. We are very grateful for her article on hypoxia-initiated DNA damage response.

In another featured article, Dr Tamzin Gristwood describes the phenomenon of Quorum Sensing, which is the mechanism by which bacteria 'talk' to each other to reach collective decisions.

We are also featuring an interview with Angela Palmer, the artist behind the *Ghost Forest*, a breathtakingly beautiful and thought-provoking exhibition that addresses the issue of rapid deforestation. It can currently be found gracing the surroundings of the Natural History Museum in Oxford.

Furthermore, Rubén Gómez Castellanos, a former employee of Lilly Mexico and current DPhil student in Chemical Biology at Oxford, talks about working in clinical research, while Eric Liu, a DPhil student in Medical Oncology gives a brief introduction to molecular epigenetics.

We're also highlighting recent publications from Oxford, accompanied by our recently introduced research comic, as well as an interview with Professor Judy Armitage, a leading name in research on bacterial flagellar motion and chemotaxis. Please also take a look at our popular science book reviews for some inspiration!

In the recently expanded Science and Society section, Alexandra East is marking the 100th anniversary of the award of the Nobel Prize in Chemistry to Marie Curie. Additionally, Stephanie Janezic discusses how the progress in stem cell research has impacted on the law, and Nicola Platt introduces us to the STEM (Science, Technology, Engineering and Mathematics) outreach programme aimed at promoting these subjects at school.

I would like to thank the *Phenotype* Team for their enthusiasm, commitment and hard work, as well as our contributing authors, without whom this issue would not have been possible. Special thanks to David Marshall, the OUBS treasurer, for his support with managing our accounts.

I hope you enjoy reading the magazine!
~Anna Boleininger

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Max-Planck-Institute for Terrestrial Microbiology, Marburg
"Regulation of cell polarity in bacteria"

Monday 7th February
Professor Amanda Fisher
MRC Clinical Sciences Centre, Imperial College London
"Stem Cells and Reprogramming"

Tuesday 8th February
12:30
OUBS annual careers day
Lecture Theatre, Medical Sciences Teaching Centre

Monday 21st February
Dr Mario de Bono
MRC LMB, Cambridge
Title to be confirmed

Monday 28th February
Professor Steve Jackson
Gurdon Institute, Cambridge
Title to be confirmed

Monday 7th March
Professor Daan Frenkel
Department of Chemistry, University of Cambridge
"DNA-coated colloids: from materials design to drug delivery?"

All seminars are held in the Main Meeting Room, New Biochemistry Building from 4 to 5 pm, unless stated otherwise.

DNA DAMAGE SIGNALLING & REPAIR

This term, OUBS welcomes Professor Steve Jackson. Professor Jackson is the Frederick James Quick Professor of Biology at the School of Biological Sciences at Cambridge University and is a senior group leader at the Wellcome Trust/Cancer Research UK Gurdon Institute of Cancer and Developmental Biology, also in Cambridge.

Over the years, Prof. Jackson's group has focussed on the interaction between different arms of the DNA damage response and how they are affected by the cell cycle. A human cell undergoes approximately 1018 DNA lesions and reparations per day. Defects in the repair process can lead to a number of problems, including premature ageing, infertility, radiosensitivity and cancer. Two important kinases involved in the sensing of DNA lesions, and in coordinating their subsequent repair, are the PI3 kinases ATM and ATR. ATM responds primarily to double strand breaks and a deficiency in this protein is responsible for the radiosensitivity syndrome Ataxia Telangiectasia (A-T). The related kinase ATR is recruited to regions of single stranded DNA following resection of double strand breaks or replication arrest. Loss of ATR is lethal to the embryo, while loss of one copy of ATR leads to Seckel syndrome, characterised by severe developmental defects.

Jackson's interest in DNA repair stems from his research into RNA splicing and regulation of transcription. He became a junior group leader in 1991 and his research into the DNA dependent protein kinase (DNA-PK) led him into the field of DNA damage signalling and repair. In 1993, Jackson and his group found that DNA-PK binds to and is activated by the ends of DNA molecules. Furthermore, they noticed that the binding properties of DNA-PK were identical to those of the autoimmune antigen Ku, whose function was yet unknown. Subsequent work in his lab led to the discovery that Ku was, in fact, the DNA-binding component of DNA-PK, mediating the recruitment of the catalytic subunit (DNA-PKcs) to DNA ends. A role for DNA-PK in the repair of DNA double strand breaks was elucidated soon afterwards with the discovery that the 80 kDa subunit of Ku (ku80) mapped to the XRCC5 gene, which was known to be involved in double strand break repair. Thereafter, Jackson and his colleagues mapped the catalytic subunit of DNA-PK to the XRCC7 gene, clarifying the role of DNA-PK in double strand break repair by the non-homologous end joining pathway (NHEJ).

The finding that the catalytic subunit of DNA-PK shared homology with the kinase domains of ATM and ATR led Jackson to study the interactions between these proteins and how their action is affected by the cell cycle. His group observed that ATR activation following a double strand break occurred later than ATM activation. Furthermore, it was seen that ATR activation only occurs in S and G2 phases of the cell cycle. This led to the hypothesis that resection of DNA ends (a critical step in homologous recombination where one strand of the DNA is 'chewed up' leaving a single stranded overhang) occurs only in S and G2 when the homologous recombination machinery can use the sister chromatid as a template for the invading DNA strand. In other phases of the cell cycle, it would be easier and less error prone for the cell to use NHEJ to repair double strand breaks. The CtIP protein works with the MRN complex to promote resection, and was found to mediate the switch between NHEJ and homologous recombination through regulatory phosphorylation events at its *C*-terminus. This was a huge leap forward in our understanding of how different DNA repair pathways work together to allow the cell to respond appropriately to DNA damage.

In 1997, Jackson founded KuDOS Pharmaceuticals (now part of Astra Zeneca). The rationale behind KuDOS was the targeting of DNA-repair pathways to increase the efficacy of radio- and chemotherapy in targeting cancer. Additionally, the targeting of these pathways would enable the selective destruction of tumour cells by exploiting the differences between these and normal cells. One very well known example of this is an inhibitor of the enzyme poly (ADP-ribose) polymerase-1 (PARP-1), which is involved in the repair of single strand breaks. In normal cells, unrepaired single strand breaks will be converted into double strand breaks in S phase, which will then be repaired by homologous recombination. Some cancers have deficient homologous recombination; in these cases, the double strand break will persist and kill the cell. This idea is currently being tested in combination with other therapies in the clinic and patients are showing a good response. The idea can be carried further still, as it is known that many cancers have DNA repair defects. Targeting repair pathways may therefore offer a means to selectively kill tumour cells while allowing normal cells to survive. Such progress would have huge implications for reducing the traumatic side effects associated with cancer treatment.

Overexpression of FTO leads to increased food intake and results in obesity

Church C, Moir L, McMurray F, Girard C, Banks GT, Teboul L, Wells S, Bruning JC, Nolan PM, Ashcroft FM, Cox RD (2010) *Nature Genetics* **42(12):1086-92.**

Up to 58% of the world's adult population is predicted to be overweight by 2030. Genome-wide association studies have revealed that single nucleotide polymorphisms (SNPs) within intron 1 of *FTO* (fat mass and obesity-associated gene) are linked to an increased risk of obesity. 16% of Caucasians are homozygous for the risk *FTO* allele and weigh on average 3 kg more than people without the allele. The molecular mechanisms behind *FTO*'s role in body weight regulation and the effect of the intron 1 SNPs are unknown.

Church *et al*. tested the hypothesis that increased levels of FTO correlate with higher body weight. They constructed transgenic mice that express three (FTO-3) and four (FTO-4) copies of the *FTO* gene, compared with two copies (FTO-2) in wild-type mice. Mice carrying additional copies of *FTO* exhibited increased body weight; for example, female FTO-4 mice weighed on average 22% more than controls at 20 weeks of age. Maintaining mice on a high-fat diet also led to increased body weight and this effect was exacerbated in FTO-3 and FTO-4 mice. Furthermore, mice overexpressing *FTO* had a higher fat-to-lean tissue mass ratio.

Human studies have indicated that individuals carrying the risk *FTO* allele demonstrate increased food intake. FTO-3 and FTO-4 mice also consumed more than controls. Furthermore, a reduction in circulating leptin, a hormone regulating food intake, was found at 8 weeks of age in FTO-overexpressing mice, though these differences were not observed in older mice. Importantly, decreased energy expenditure was ruled out as a cause of obesity in FTO-3 and FTO-4 mice. In conclusion, this study shows that enhanced expression of *FTO* leads to increased fat mass and obesity most likely caused by increased food intake. Though this does not necessarily recapitulate the situation in humans, it does suggest that the risk *FTO* human allele might increase the expression of FTO or enhance its activity. Excitingly, this work may aid the development of anti-obesity drugs, which reduce appetite via decreased *FTO* activity.

Break-induced ATR and Ddb1-Cul4^{Cdt2} ubiquitin ligase-dependent nucleotide synthesis promotes homologous recombination repair in fission yeast

Moss J, Tinline-Purvis H, Walker CA, Folkes LK, Stratford MR, Hayles J, Hoe KL, Kim DU, Park HO, Kearsey SE, Fleck O, Holmberg C, Nielsen O, Humphrey TC (2010) ***Genes & Development*** **24:2705-16.**

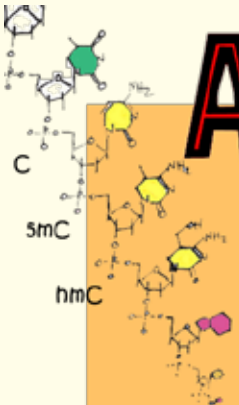
DNA double-strand breaks (DSBs) are dangerous lesions that, if left unrepaired, can cause chromosomal rearrangements, cancer, or cell death. The homologous recombination (HR) repair pathway, in which a homologous DNA sequence from the sister chromatid is used as a template for repair, exists in cells to maintain genome integrity. Synthesis of nucleotides by the enzyme ribonucleotide reductase (RNR) is required for DNA repair; indeed, nucleotide synthesis is a universal response to DNA damage. Precisely how nucleotide synthesis is increased in response to damage has remained unclear.

Using a genetic screen, Moss *et al*. have found that the Ddb1-Cul4^{Cdt2} ubiquitin ligase complex, together with RNR, is required for HR repair following a DSB in fission yeast. Both *ddb1Δ* and *cdt2Δ* mutant strains were sensitive to damaging agents and were unable to undergo HR repair following bleomycin-induced DSBs. The Ddb1-Cul4^{Cdt2} ubiquitin ligase complex targets Spd1 (an inhibitor of RNR) for degradation. As predicted, deleting *spd1* suppressed the DNA damage sensitivity and the reduced HR efficiency associated with loss of *ddb1* or *cdt2*.

Furthermore, Moss *et al*. showed that after DNA damage, Cdt2 nuclear levels are increased, thereby stimulating nucleotide production for DSB repair. Intriguingly, nuclear accumulation of Cdt2 is dependent on the damage-sensing kinase Rad3 (ATR). Thus, Moss *et al*. reveal a model in which activated Rad3 heads a Ddb1-Cul4^{Cdt2}-dependent pathway leading to increased nucleotide pools that can then fill single-stranded DNA gaps, thereby repairing DSBs.

A Tale of Two Strands

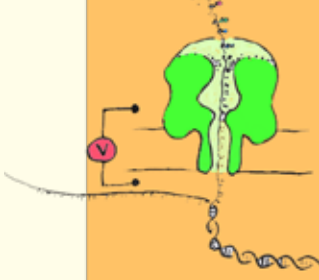
Fresh DNA research from Oxford scientists!



DNA consists of the four bases ATCG. In addition, C can be modified to give 5-methyl C (5mC) and 5-hydroxy-methyl C (hmC). Such examples of epigenetics control gene expression and the pattern changes throughout development. Epigenetics is the prime suspect for why cloning suffers a low success rate.

In the CRL, the Bayley group addresses **epigenetic sequencing** using nanopore technology.

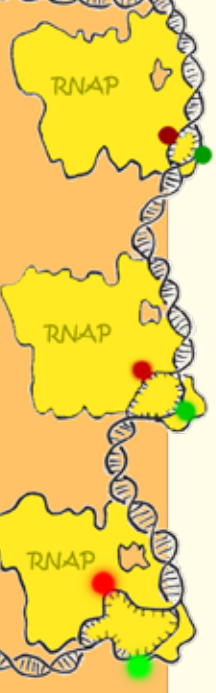
Single-stranded DNA is immobilised in a membrane-situated protein pore and a voltage is set up across the membrane. As ions try to cross the pore, the DNA bases in their way determine the resistance they meet. Consequently, the current profile reveals the DNA sequence. For instance, the obstructing 5mC methyl group results in 8.5% less current, allowing 5mC and C to be distinguished.



Wallace EV, Stoddart D, Heron AJ, Mikhailova E, Maglia G, Donohoe TJ, Bayley H. (2010) Identification of epigenetic DNA modifications with a protein nanopore. Chem Commun (Camb) 46(43):8195-7 PMID: 20927439

Meanwhile, in the department of Physics, the biological physics group report an application of **Förster Resonance Energy Transfer** (FRET) to measure size and kinetics of the DNA transcription bubble.

In FRET, two particles fluoresce depending on the distance between them. The group adapted FRET to quench fluorescence if particles are within 2 nm, whereas larger particle inter-distance increases individual fluorophore transmission. They nicknamed it quFRET. Then they put one quFRET particle on each DNA strand of the lac promoter and let bacterial RNA polymerase (RNAP) transcribe.

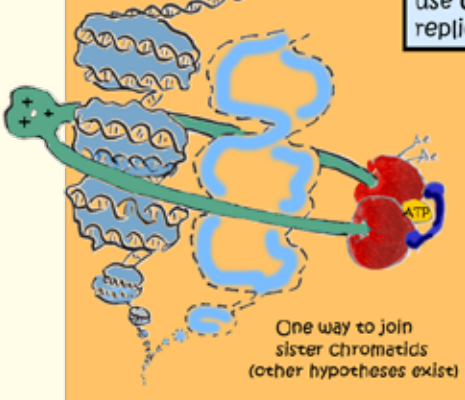


Their results verify previous research findings on promoter opening kinetics and on what stage of transcription RNAP inhibitors act. However, quFRET needs additional calibration to measure the distance between strands as they transit through RNAP.

In the future, quFRET may be used to investigate proposed models of transcription or screen for antibiotics that target bacterial transcription.

Cordes T, Santoso Y, Tomescu AI, Gryte K, Hwang LC, Camará B, Wigneshweraraj S, Kapanidis AN. (2010) Sensing DNA opening in transcription using quenchable Forster resonance energy transfer. Biochemistry 49(43):9171-80. PMID 20813825

Each human cell contains 2m of DNA. Therefore, after replication, it contains 4m. To keep this DNA untangled, cells use **cohesin complexes** that associate with chromatin before replication, and subsequently link sister chromatid strands.



One way to join sister chromatids (other hypotheses exist)

Cohesins resemble cherries with a hinge, twigs and two large ATP-binding domains (NBD) that glue together upon hydrolysing ATP or when joined by a linker molecule. It is unknown how cohesin joins sister chromatids, but lack of NBD acetylation or chemical linkage of the hinge prohibit joining. As does hinge neutralisation, as Oxford biochemists have discovered. The group crystallised the mouse hinge and found that it resembles the bacterial hinge crystal structure, both in terms of circularity and the positively charged cylinder interior. Modelling suggests that this curious charge distribution is conserved across several eukaryotes and prokaryotes. To investigate the purpose of the charge, they neutralised it and observed the result phenotype assisted by fluorescent tags.

Dimerisation, structure, kinetics and cellular distribution were all normal. Association to DNA was slightly too stable. More strikingly, neutralising the hinge interior results in 5-6 times less NBD acetylation, and sister chromatids are never joined, which causes spindle poles to be 50% more separated than they should be, and the cell to die.

The authors of the paper hypothesise that hinge opening flaunts otherwise concealed positive charge, allowing acetylation of the NBD, possibly by association with the hinge and ATP hydrolysis.

Maybe the extent of hinge opening could be investigated by quFRET?

Kurze A, Michie KA, Dixon SE, Mishra A, Itoh T, Khalid S, Strmecki L, Shirahige K, Haering CH, Löwe J, Nasmyth K. (2010) A positively charged channel within the Smc1/Smc3 hinge required for sister chromatid cohesion. EMBO J PMID: 21139565

by Caroline Dahl

The hypoxia-induced DNA damage response

Dr Ester Hammond

For cells to form tumours, they must adapt to living in unique conditions, collectively referred to as the tumour micro-environment. These conditions include low oxygen (hypoxia). Cancer cells which successfully adapt to life with limited oxygen become more aggressive and resistant to therapy. Research into the cellular response to hypoxia is therefore crucial to the understanding of tumourigenesis. We study the DNA damage response which is induced by exposure to hypoxia, with the goal of exploiting this process for therapeutic gain.

Why does hypoxia occur in tumours?

Put simply, regions of hypoxia occur in solid tumours because the demand for oxygen exceeds the available supply. Areas of chronic hypoxia arise because there are so many tumour cells actively metabolising oxygen that those cells that are a distance away from a blood vessel become starved of oxygen. Tumour hypoxia exists as a gradient of oxygen tensions from normal (normoxic) adjacent to the blood vessel to anoxic (no oxygen) further from the vessel. Chronically hypoxic or anoxic cells are found surrounding necrotic (dead) areas of the tumour.

One of the ways tumour cells adapt to hypoxia is by trying to restore oxygen/nutrient levels through angiogenesis. Angiogenesis is the formation of new blood vessels from existing vessels and is one of the original six hallmarks of cancer. To a large extent, angiogenesis is controlled by the hypoxia inducible factor 1 (HIF1) transcription factor. Although tumours are able to create new vasculature and therefore improve oxygen delivery, the tumour vasculature is poorly formed and inefficient compared with normal vasculature. This results in blockages and leaks in the vessels and fluctuations in the surrounding interstitial fluid, which can lead to the build up of acutely hypoxic regions. Acutely hypoxic areas can undergo rapid reperfusion or reoxygenation as a result of the opening of a transiently occluded blood vessel. Because reoxygenation also induces a biological response, hypoxia and reoxygenation are often considered two facets of a single stress (1).

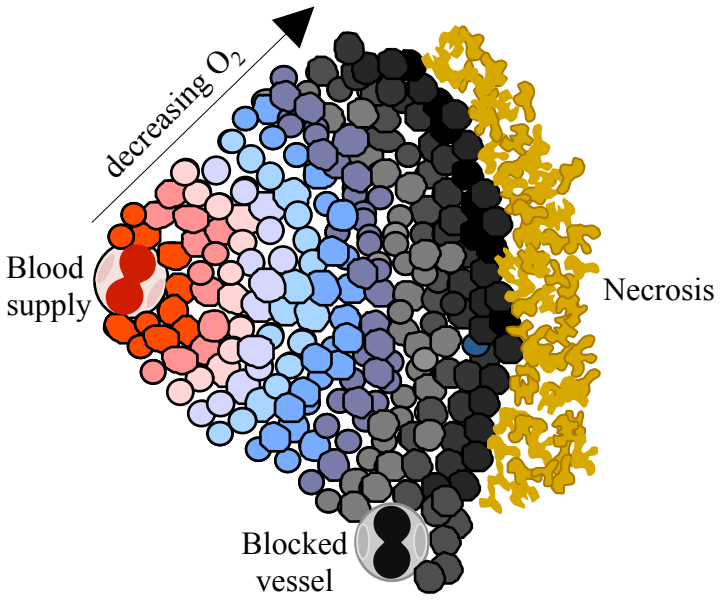


Figure 1. Chronic versus acute hypoxia in tumours. Areas can be either chronically hypoxic due to distance of the cells from a functional vessel, or acutely hypoxic due to a blockage, which may be transient, in a nearby vessel.

Relevance of tumour hypoxia to therapy

Regions of hypoxia occur to some degree in most solid tumours, irrespective of the organ of origin. This is significant as these hypoxic cells become more aggressive and more resistant to therapy. Once a cell has adapted to hypoxic conditions, it is far more likely to metastasise and acquire further mutations which may increase its growth potential further. Therefore, for therapy to be curative it is imperative that all hypoxic cells are removed.

The ability to treat cancer with chemotherapy relies on delivering these drugs to the tumour cells. However, if these cells exist in an area which is not perfused, the drugs will not be delivered and, in some cases, the drugs are not as potent in the absence of oxygen. Most significant is the finding that hypoxic cells show increased resistance to radiation. It has been clearly demonstrated that hypoxic cells are up to three times more resistant to radiation than normal cells under typical oxygen levels. In order to eradicate all hypoxic cells, three times the dose of radiation required to kill normal cells which, of course, surround the tumour, must be delivered.

What is the DNA damage response?

The DNA damage response (DDR) is a term used

to group a complex series of signalling pathways which are initiated in response to various forms of DNA damage (2). In general, a signal such as a double strand break or a missing base is detected by sensor complexes, leading to the activation of one of the PI3-kinases (e.g. ATM, ATR and DNA-PK). This signalling generally results in activation of the cell cycle checkpoints to promote cell cycle arrest and allow time for DNA repair, although cell death is another possible outcome. During the process of tumourigenesis, cancer cells often lose one or more of their DNA repair pathways and must rely on redundancy between the repair pathways.

Many forms of cancer therapy are based on the ability to induce large amounts of DNA damage. Normal cells, which possess a full complement of repair pathways, are able to repair the damage. However, tumour cells, in which some repair pathways have been disabled for example by mutation, are unable to repair themselves and die. Components of the DDR have therefore become increasingly attractive therapeutic targets as inhibition of the DDR further diminishes the ability of the cancer cell to survive therapy-induced DNA damage. There are a number of clinical trials in progress using inhibitors of the DDR in combination with standard therapies.

The hypoxia-induced DDR

It has been demonstrated that severe levels of hypoxia induce a DNA damage response characterised by both ATM and ATR signalling. This is somewhat surprising as it seems that the hypoxia-induced DDR occurs in the absence of DNA damage. Recently, our group demonstrated that hypoxia belongs to the group of stresses known to induce a replication arrest and subsequent DDR. In response to severe hypoxia, the activity of the enzyme responsible for nucleotide production is reduced, leading to a decrease in available nucleotides. Without these building blocks, DNA is no longer synthesised and a replication arrest occurs. This is then sensed by ATR, which initiates the hypoxia-induced DDR. Recent studies suggest that additional mechanisms for activating the DDR in response to hypoxia exist, as activation has been demonstrated in specific cell types in the absence of replication arrest (3).

Hypoxia is one of very few stresses that activates ATM in the absence of DNA damage and is therefore of significant therapeutic interest (4, 5). It has been demonstrated that inhibition of components of the DDR, such as the checkpoint kinases Chk1 and Chk2, sensitises cells to hypoxia/reoxygenation.

Hypoxia and DNA repair

All of the five major DNA repair pathways are repressed under hypoxic conditions (6). The biological reason or benefit to shutting down DNA repair in response to hypoxia is unclear and is the subject of much debate. What is clear is that the cell has

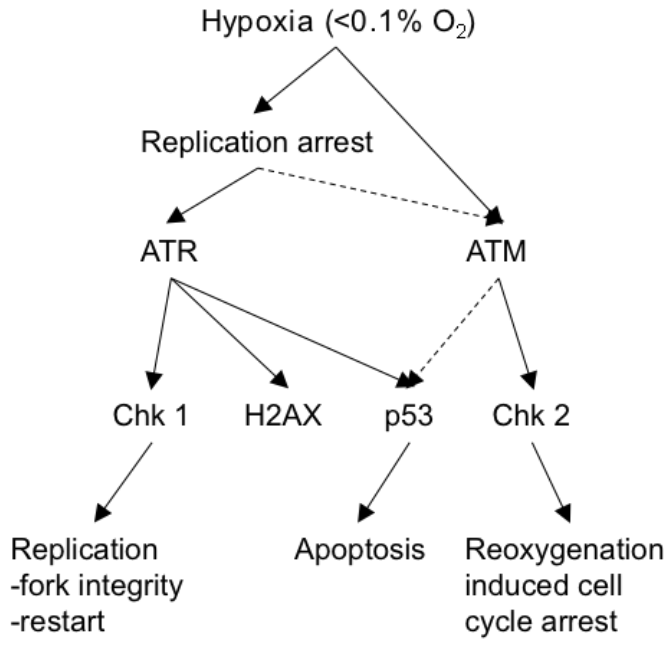


Figure 2. The hypoxia-induced DDR. Both ATM and ATR respond to hypoxia in an oxygen-dependent manner and subsequently phosphorylate downstream targets as shown.

multiple mechanisms in place to ensure repair pathways are switched off in hypoxia, including repression of certain proteins by HIF1 and increase in specific micro-RNA (miRNA) activity. The finding that DNA repair is repressed by hypoxia has led to the hypothesis that hypoxic cells might be more sensitive to treatment with DDR or DNA repair inhibitors. We are interested in exploring this possibility and have focused on the hypoxia-mediated repression of homologous recombination (HR).

HR, one of the multiple DNA repair pathways, responds to double strand breaks. Cancer cells can tolerate a loss of HR because of other mutations they acquire, for example loss of p53. BRCA1 plays a pivotal role in HR and this gene is mutated in approximately 10% of breast cancers. In contrast, the protein PARP is involved in the repair of single strand breaks (SSBs). However, if a SSB is unrepaired due to the inhibition of PARP, it is processed to form a double strand break during DNA replication and is subsequently repaired by HR. This led to the use of PARP inhibitors to treat BRCA1 mutated tumours as in the absence of both repair pathways the cell is killed. This is termed synthetic lethality and is an increasingly attractive strategy for cancer therapy (7, 8). Our hypothesis was that since hypoxic cells repress HR they should be sensitive to PARP inhibition. We have recently validated this hypothesis, extending the possible use of PARP inhibitors from agents to treat tumours with known HR mutations to being considered for the treatment of any hypoxic tumour (9). The combination of hypoxia-mediated repression of HR and PARP inhibitors to kill cancer cells is referred to as 'context synthetic lethality'.

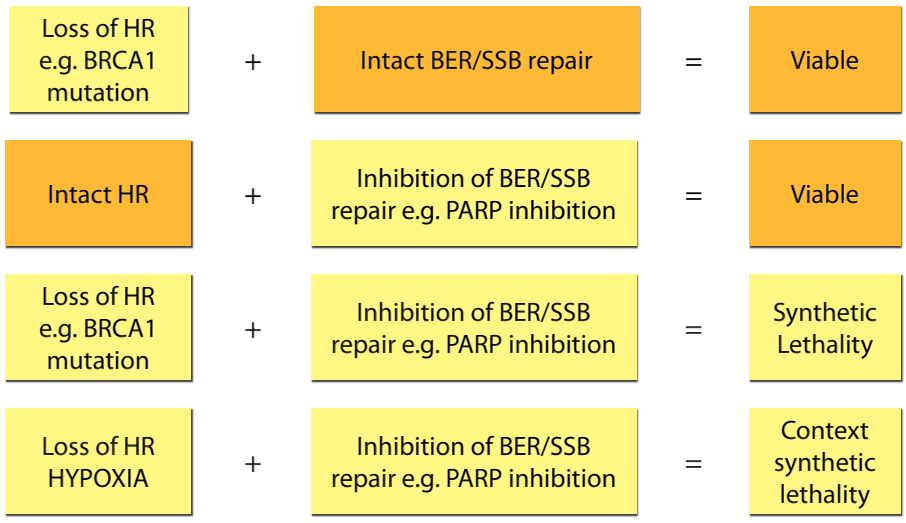


Figure 3. Context synthetic lethality. Loss of homologous recombination (HR) or base excision repair (BER) can be tolerated in human tumours but loss of both results in synthetic lethality i.e. the cell dies. In the context of hypoxia, HR is also repressed and consequently the cells become sensitive to PARP inhibition.

Conclusions

Environmental adaption is a common feature of cells. Primary cells isolated from humans adapt to the standard laboratory conditions used which bear little resemblance to those experienced in the body. In the same way, cancer cells adapt to the hypoxic tumour micro-environment and, by hijacking developmental pathways like angiogenesis, thrive. Therefore, it is imperative that cancer therapy targets the hypoxic fraction of tumours. Some promising examples already in clinical trials include the use of HIF inhibitors and anti-angiogenesis compounds.

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7. Bryant HE, *et al.* (2005) Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase. *Nature* 434 (7035):913-7.

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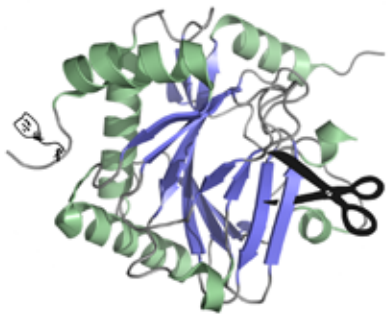
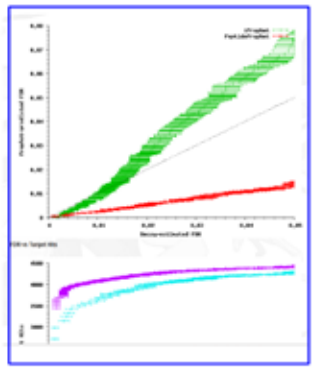
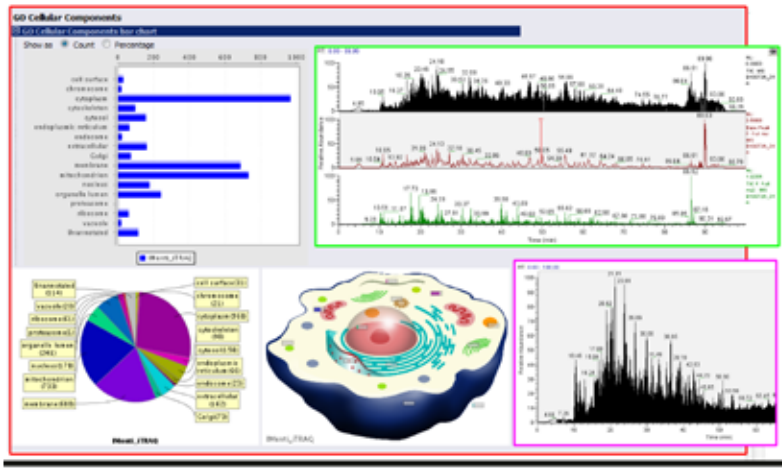
9. Chan N, *et al.* (2010) Contextual Synthetic Lethality of Cancer Cell Kill Based on the Tumor Microenvironment. *Cancer Res* 70(20):8045-54.

Dr Ester Hammond is a Cancer Research UK Junior Group Leader at the Gray Institute for Radiation Oncology and Biology, University of Oxford.

Oxford Central Proteomics Facility







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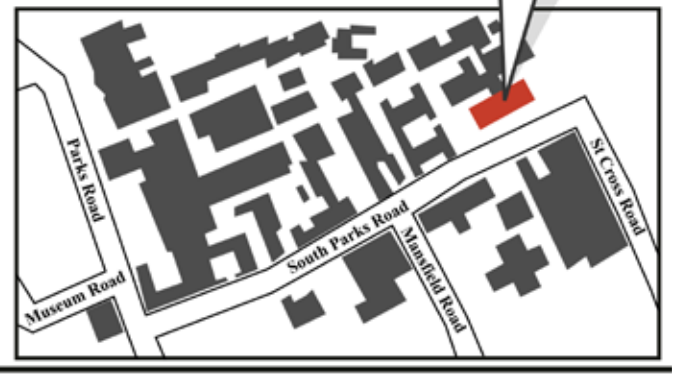

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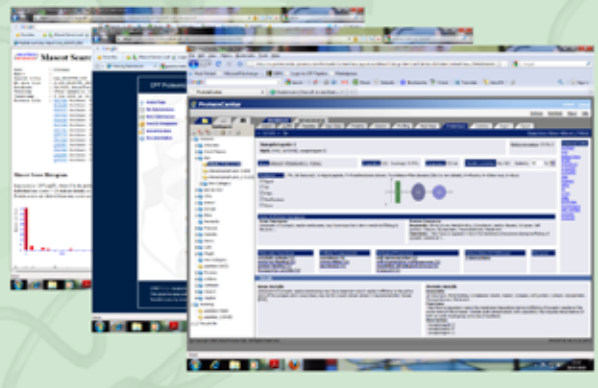
- Identification of complex protein mixtures by GeLC-MS (tens to thousands of proteins can be identified)
- Identification and localisation of post-translational modifications (phosphorylation, acetylation, methylation, ubiquitination, oxidation etc)
- Quantitative proteomics: compare protein expression levels in whole-cell lysates using isotopic labelling techniques (SILAC, iTRAQ)
- Label-free quantitation: compare protein expression levels between primary cells, patient tissue and serum *without* isotopic labelling

Instrumentation Available

- Thermo LTQ Orbitrap LC-MS/MS mass spectrometer coupled to a Dionex U3000 nano HPLC system
- Applied Biosystems 4800 MALDI TOF/TOF mass spectrometer with LC-MALDI capability

Software Available

- Mascot, ProteinCenter, Central Proteomics Facility data analysis pipeline, label free quantitation software



Clinical Research and its Career Prospects

J. Rubén Gómez Castellanos

J. Rubén Gómez Castellanos, currently a DPhil student in the Department of Chemistry, previously worked as a Junior Clinical Research Associate at Eli Lilly and Company, Mexico. Based on his experience within the pharmaceutical industry, he explains the processes involved in licensing new drugs and describes what the job of a Clinical Research Associate entails.

The pharmaceutical industry is the main sponsor of medicine research worldwide. Sponsors must demonstrate the safety, quality and efficacy of potential new medicines – called an investigational medicinal product (IMP) in the UK, and New Chemical Entity (NCE) in the USA – through rigorous clinical trials. A licence can then be obtained, allowing doctors to prescribe the drug (1).

The ICH* define a clinical trial as "any investigation in human subjects intended to discover or verify the clinical, pharmacological and/or other pharmacodynamic effects of an investigational product(s), and/or to identify any adverse reactions to an investigational product(s), and/or to study absorption, distribution, metabolism, and excretion of an investigational product(s) with the object of ascertaining its safety and/or efficacy."(2)

The importance of having an independent evaluation of any new medicinal product was realised at different times in different regions. In the United States the trigger for setting up the product authorisation system under the Food and Drug Administration (the Food, Drug and Cosmetic Act, 1938) was the Massengill incident of 1937, in which an improperly prepared elixir of sulphanilamide with diethylene glycol caused the deaths of more than 100 people (3). In many countries in Europe the trigger was the thalidomide tragedy of the late 1950s, which revealed that the new generation of synthetic drugs had the potential to harm as well as heal (4).

The Good Clinical Practice Guideline (GCPs) is an ICH directive describing the responsibilities and expectations of all people involved in conducting clinical trials, including investigators, monitors, sponsors and institutional review boards. GCPs cover aspects of monitoring, reporting and archiving of clinical trials.

Clinical trials in humans

Clinical trials in humans are divided into four phases, which in practice often overlap (Table 1). Phases I to III are done before a licence is granted and Phase IV occurs after authorisation. The

Phase	Subjects	Data
I	50-200 Healthy subjects or patients	Safety in healthy individuals Pharmacokinetics Pharmacodynamics Toxicology Safety Dosage Range
II	100-400 Patients with the target disease	First Effective Dose Safety in patients
III	1000-5000 Patients with the target disease	Confirmation of effectiveness Monitoring of side effects Regulatory data for registration
IV	Ranging from several thousand to several million patients with the target disease	Long term safety (pharmacovigilance)

Table 1. Phases in Clinical Research (1).

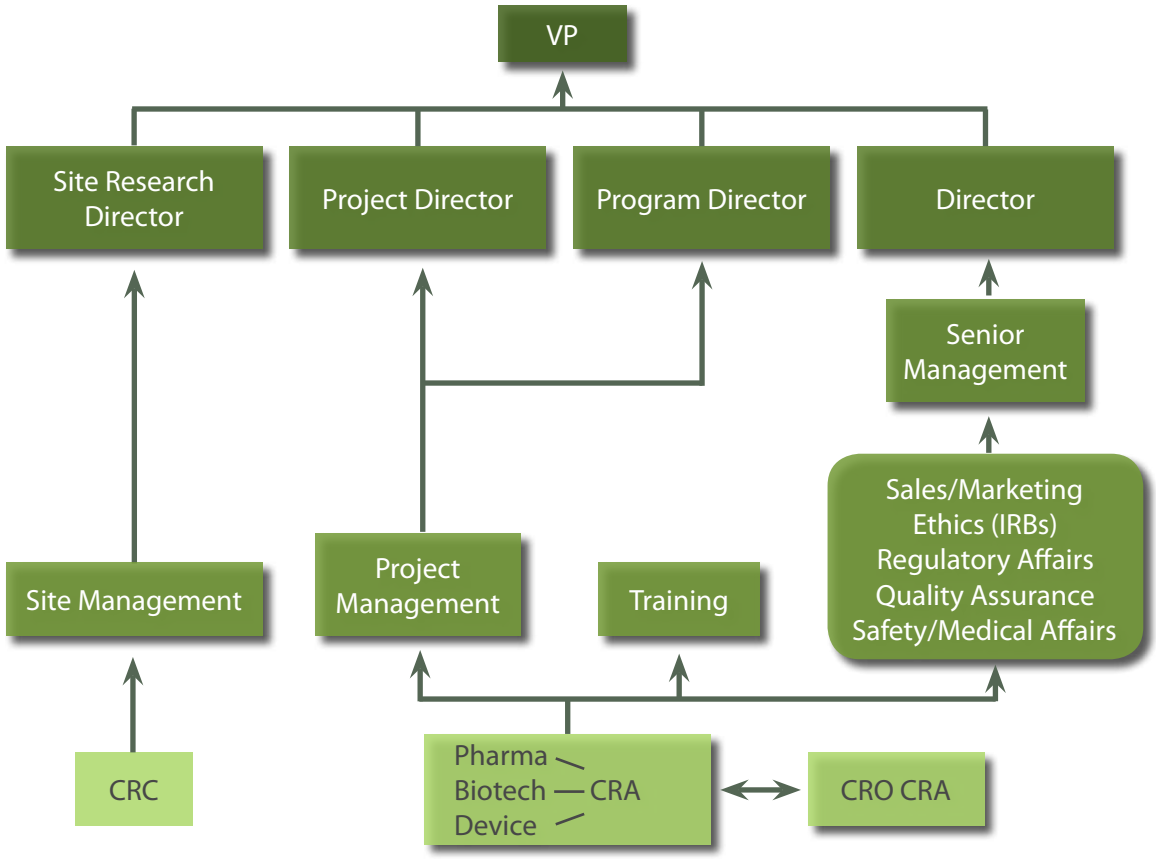


Figure 1. The Clinical Research Career Ladder. Adapted from (6).

phases differ in terms of the number and types of subject studied, and the questions asked (1).

Pharmaceutical companies use drug development money to finance the vast majority of clinical studies: bringing new medicines from bench to bedside. Many of these trials are rigorously designed, employing the skills of outstanding clinical researchers at leading academic institutions. But academic medical centres are no longer the only institutions being employed for clinical research. The past 20 years have seen the growth of commercially oriented networks of contract-research organisations (CROs) and site-management organisations (SMOs). This has altered the drug-trial landscape, forcing academic medical centres to rethink their participation in industry-funded drug research (5). This is in line with GCPs which state that "a sponsor may transfer any or all of the sponsor's trial-related duties and functions to a CRO, but the ultimate responsibility for the quality and integrity of the trial data always resides with the sponsor. The CRO should implement quality assurance and quality control." (2)

This clinical trial model has opened a market that for 2010 has been estimated to have revenues of $20 billion USD, approximately one third of total research and development spending across the pharmaceutical and biotechnology sector (6). This has provided numerous job opportunities for science graduates all over the globe.

As mentioned above, the design, and hence the quality, of a clinical trial remains the responsibility of the pharmaceutical company sponsoring the trial. The responsible team usually consists of physicians (often referred to as Clinical Research Physicians), pharmacists, pharmacologists, statisticians and senior management overseeing a product or a therapeutic area. Nowadays, in order to avoid any conflict of interest or bias, the marketing team is generally not involved in the running of clinical trials.

The role of the Clinical Research Associate

Monitoring is a critical part of clinical trial execution. The purpose of monitoring is to verify that the rights and well-being of human subjects are protected and to ensure that the data are accurate, complete, and verifiable from source documents. In addition, it must be ensured that the conduct of the trial complies with the currently approved protocol/amendment(s), with GCP, and with the applicable regulatory requirements (2). The member of the team responsible for this monitoring is the Clinical Research Associate (CRA, or Monitor).

The CRA position is the entry-level position of choice in any clinical research organisation. The main tasks of a CRA are listed in Box 1. In spite of the perceived specialised nature of the position, it is a very versatile one, which facilitates future movements within the organisation. The usual progression of a CRA within the organisation can be seen in Figure 1. In addition to the CRA position (either in a pharmaceutical, biotechnological or medical device company, or in a CRO), Figure 1 shows the position of the Clinical Research Coordinator (CRC, the counterpart of the CRA in the research site). It is not uncommon for a person to interchange between

The main tasks of a CRA are:

- Liaise between the Sponsor and the Investigator
- Verify Investigator's (and staff's) qualifications before and during the trial
- Manage the Investigational Product
- Assure protocol compliance
- Verify that informed consent was obtained
- Check all documentation is complete, filed and up to date
- Perform source data verification
- Document serious adverse events

the two positions before settling on a specific role. A CRA can expect to spend three to five years developing their skills as a CRA before making their first upward movement within the company. If the candidate decides to stay in clinical research, this would usually mean a career in the Medical Division of the organisation; in the best case scenario and given the right credentials, including a medical degree, this might lead to the position of Medical Director. Alternatively, as shown in Figure 1, a CRA might instead transfer to diverse departments within the company.

In conclusion, a career in clinical research can act as an excellent entry into the pharmaceutical/ CRO industry with excellent prospects for advancement within the clinical research organisation or other areas of the company.

Footnote

* The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH)

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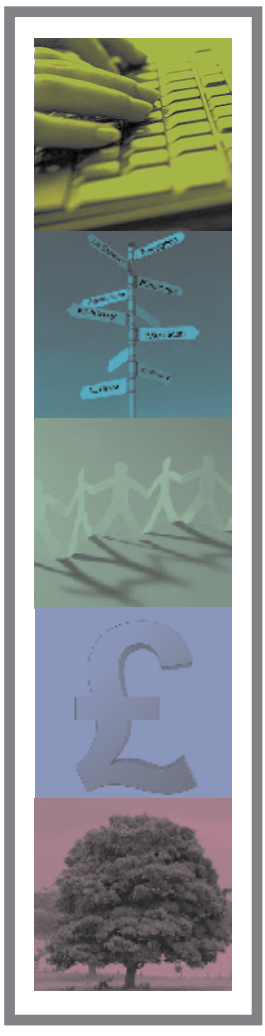

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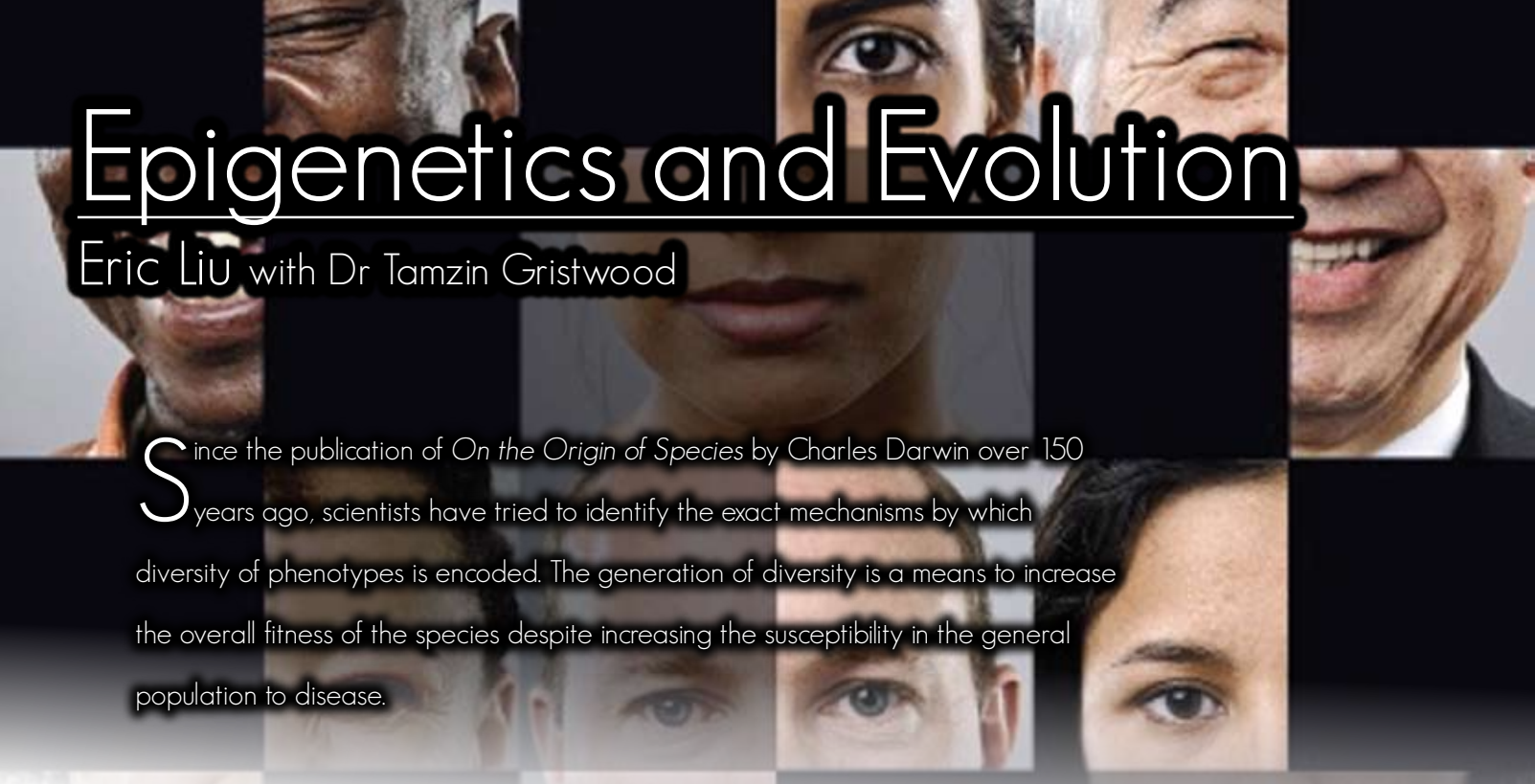

Epigenetics and Evolution

Eric Liu with Dr Tamzin Gristwood

Since the publication of *On the Origin of Species* by Charles Darwin over 150 years ago, scientists have tried to identify the exact mechanisms by which diversity of phenotypes is encoded. The generation of diversity is a means to increase the overall fitness of the species despite increasing the susceptibility in the general population to disease.

Since the identification of the genetic code, the classical view has been that this variability was encoded by the DNA sequence. The identification of single nucleotide polymorphisms (SNPs) was highly anticipated when the human genome was sequenced. However, despite definitely forming part of the explanation of diversity, SNPs did not explain everything. Genome-wide association studies revealed SNPs could only account for 10 to 20% of the variability in phenotypes such as height (1). Therefore many of the sources of diversity remain elusive.

Lamarck's theory

Just over 200 years ago, in the same year as Darwin's birth, the French biologist Jean-Baptiste Lamarck published his own theory of evolution. Until recently, his theory was considered inaccurate. Lamarck proposed that individuals can develop traits that are useful during their lifetime and pass these on to their offspring (3). Traits that are not useful would be lost from the population. An example would be the idea that, by constantly stretching their necks to reach food, a giraffe would lengthen and strengthen its neck and this trait would be passed to its offspring. This notion of acquired ('soft') inheritance contrasted with Darwin's theory of natural selection, which diminished the importance of individual efforts in adaptation to the environment.

Epigenetics

The term epigenetics was coined by the British scientist C. H. Waddington in 1942 (4). Waddington's description of epigenetics came before the molecular basis of genes and heredity was understood, thus he referred to a more general concept that would allow genes to interact with their environment to generate a specific phenotype. Today, epigenetics refers to heritable traits that do not involve changes to the actual sequence of DNA.

As epigenetic changes can be acquired through environmental pressures, for example diet, sunlight, or viral infection, people started to reconsider the principle of Lamarckian inheritance. In addition to Lamarckian inheritance, another theory, termed non-Lamarckian inheritance, has recently been proposed to be mediated via epigenetics. This article examines the role of epigenetics in both of these theories.

The molecular basis of epigenetics

DNA methylation and chromatin remodelling, via modification of histones, allow gene expression to be altered without changing the DNA sequence. DNA methylation involves the addition of methyl groups to cytosines, mainly at CpG sites. This methylation pattern, which influences gene expression, can be inherited when cells divide.

Histone proteins, which together with DNA form chromatin, can be post-translationally modified. This includes covalent modifications by acetylation, methylation, ubiquitylation, phosphorylation and SUMOylation. Commonly, but not exclusively, these modifications occur within the unstructured *N*-terminal histone tails. Modification of histones can result in changes to chromatin structure and hence changes in gene expression. It can also alter binding of other proteins to DNA, for example transcriptional regulators. As histones remain associated with DNA during cell division, histone-mediated changes in gene expression can be heritable.

MicroRNAs can act to enhance or repress expression of target genes. As RNA, along with protein, is transferred to the daughter cell during cell division, microRNA-mediated gene expression effects can also be inherited.

Finally, prions have been found to play a role in epigenetic inheritance in yeast. Prions operate by

affecting protein folding, resulting in different conformational states of the same protein which can have very different functions. Prions replicate by templating the conformational conversion of other molecules of the same protein to the prion-protein form. Thus, the action of prions can diversify protein functions and can even generate a change in function. Prions can be transferred to the daughter cell during cell division and direct proteins to fold into the prion form, thus the resulting phenotypic trait would be heritable. However, although prions have been identified as disease-causing agents in humans and other mammals, a role in human inheritance has not yet been established.

Epigenetics in Lamarckian inheritance

Epigenetics plays a fundamental role in the development of multicellular organisms, allowing cells with the same DNA content to differentiate and form distinct tissue types. Previously, it had been thought that gamete cells were not susceptible to epigenetic changes, hence the epigenetic patterns would be 'reset' when the organism reproduced. There is now a wealth of experimental evidence indicating that epigenetic patterns can be transmitted from parents to their offspring – transgenerational epigenetics. For example, in one study, gestating female rats were exposed to endocrine disruptors, resulting in decreased spermatogenic capacity and increased incidence of infertility in male offspring (3). It was discovered that certain regions of DNA in the sperm from the male offspring were hypermethylated. This methylation pattern, and hence the reduced-fertility phenotype, was transferred to the male germ lines over multiple generations. Further evidence came from a study in which stressed mother rats, which spent less time caring for their offspring, produced offspring with a fear phenotype similar to that of the mother (3). This behaviour persisted into adulthood.

Transgenerational epigentics can be considered to follow Lamarck's theory of evolution. However, it has been argued that epigenetics simply allows diverse phenotypes within a population to be reversibly exhibited rather than actually providing a means to introduce novelty into a species. Thus, the importance of epigenetics in the evolution of a species remains questionable.

Non-Lamarckian inheritance and epigenetics

While much of the focus has been on Lamarckian inheritance, A. Feinberg and R. Irizarry at John Hopkins University proposed a non-Lamarckian model of inheritance to incorporate the effect of environmental exposures on evolutionary selection (5). While not disputing Lamarckian inheritance, this group proposed that certain epigenetic variations act to increase heterogeneity in a species in order to promote survival of the species following a change in the environment. They examined methylation patterns in brain and liver samples from mice that had been raised under identical conditions. Despite the identical environment, specific regions of DNA showed highly variable levels of DNA methylation.

In addition, mathematical modelling was used to investigate the likelihood of a species becoming extinct over 1000 generations. Using a hypothetical phenotype (Y) which affected survival, they demonstrated that although a variable Y trait was detrimental in a static environment, it was highly beneficial when the species was exposed to multiple environmental changes. Thus, an increase in phenotype variability, even without a change in the mean phenotype, can significantly increase the overall fitness of a species. Ultimately, there will be a balance between the generation of diversity and the production of viable and fertile offspring.

Conclusion

As our understanding of cellular processes increases, so too does our understanding of inheritance. The mechanisms of epigenetic regulation offer exciting ways for nature to increase phenotypic diversity and variability. It should be noted that the role of epigenetics in phenotypic variation allows both Darwinian evolution and acquired inheritance theories to be valid. Future advances in our understanding of epigenetics will help us to better explain mechanisms of inheritance and how they drive evolution.

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All Talk

Dr Tamzin Gristwood

In November 2010, Professor Bonnie Bassler from Princeton University gave the second Joel Mandelstam Lecture in Oxford. Professor Bassler is a well-known personality in the field of Quorum Sensing (QS), the mechanism by which bacteria 'talk' to each other. It was while attending one of her characteristically energetic seminars during my undergraduate degree that I was inspired to do my PhD in the QS field, a topic I had not even heard of before attending that seminar! Professor Bassler certainly managed to convey to me the wonder of the mechanisms – including QS – which bacteria use to adapt effortlessly to the constant and often extreme changes in their environment. This article explains what QS is, highlights some of the fascinating ways that bacteria employ QS, and discusses some of the potential applications of QS research.

What is Quorum Sensing (QS)?

QS describes the mechanism by which bacteria are able to communicate with each other via the production of low molecular weight signalling molecules in order to coordinate group behaviour in response to cell density (1).

The first QS system to be characterised, and now considered the paradigm of QS in Gram-negative bacteria, is that of *Vibrio fischeri* (2, 3). This bioluminescent (light producing) bacterium forms a symbiotic relationship with the squid *Eupryma scolopes* by colonising a specialised light organ. The light produced by *V. fischeri* allows the squid to counteract the shadow it casts when hunting, thus allowing it to remain unobserved by its prey. In return, the squid provides a nutrient-rich environment in which the bacteria grow. However, *V. fischeri* can also be found free-living in the marine environment, where it does not produce light.

It was discovered that *V. fischeri* cells only express the genes required for light production, located on the luciferase operon, when growing at high cell density, such as when contained within the light organ of the squid or indeed a flask in the laboratory. Further research revealed that *V. fischeri* produces freely diffusible signalling molecules which accumulate in the extracellular environment. These signalling molecules, acyl homoserine lactones (AHLs), are produced by the constitutively active AHL-synthase, LuxI. Thus, when growing within a confined volume, the extracellular AHL concentration is directly proportional to the number of *V. fischeri* cells present. When the AHL concentration reaches a minimum threshold, it is detected via the LuxR transcriptional regulator. LuxR binds to cytoplasmic AHL and the resulting LuxR-AHL complex activates transcription of the luciferase operon, producing light. It is presumed that as the energetic process of producing light would be futile when very few *V. fischeri* cells are present, the bacteria only produce light when there are enough cells present to result in significant light production.

Variations on the same theme

Although all QS systems identified thus far are analogous, the nature of the signalling molecule produced (collectively called autoinducers), the specific proteins involved in detecting the signal, and the phenotypic output can vary. For example, Gram-positive bacteria typically use modified oligopeptides as autoinducers. Unlike the freely diffusible AHLs, the oligopeptide autoinducers usually require an export system to cross the cell membrane. In addition, rather than being detected by cytoplasmic LuxR, oligopeptide autoinducers are detected by a membrane-bound receptor. This signal is transduced via a series of phosphorylation events within the cell, ultimately resulting in changes in expression of the target genes. Other types of autoinducers include fatty acids, fatty acid esters and 4-quinolones.

The range of bacterial phenotypes regulated by QS is immense. For example, QS controls expression of virulence factors in the human pathogen *Staphylococcus aureus*, and transfer of the tumorgenic Ti plasmid from *Agrobacterium tumefaciens* to the plant host. The plant pathogen *Erwinia carotovora* not only uses QS to regulate production of plant-cell-wall-degrading exoenzymes but also the production of a β-lactam antibiotic, thus preventing its competitors from enjoying the resulting plant-derived feast. Other phenotypes include motility, biofilm formation, adhesion, competence for DNA uptake and sporulation.

Many bacteria, including the opportunistic human pathogen *Pseudomonas aeruginosa*, possess multiple QS modules which are often interdependent,

forming complex hierarchical regulatory networks. To make matters even more complicated, regulation in response to other environmental cues, for example temperature, carbon source or oxygen availability, is commonly integrated into the QS system, allowing the bacteria to thoroughly assess their surroundings and coordinate the most appropriate gene response.

Interspecies and interkingdom communication

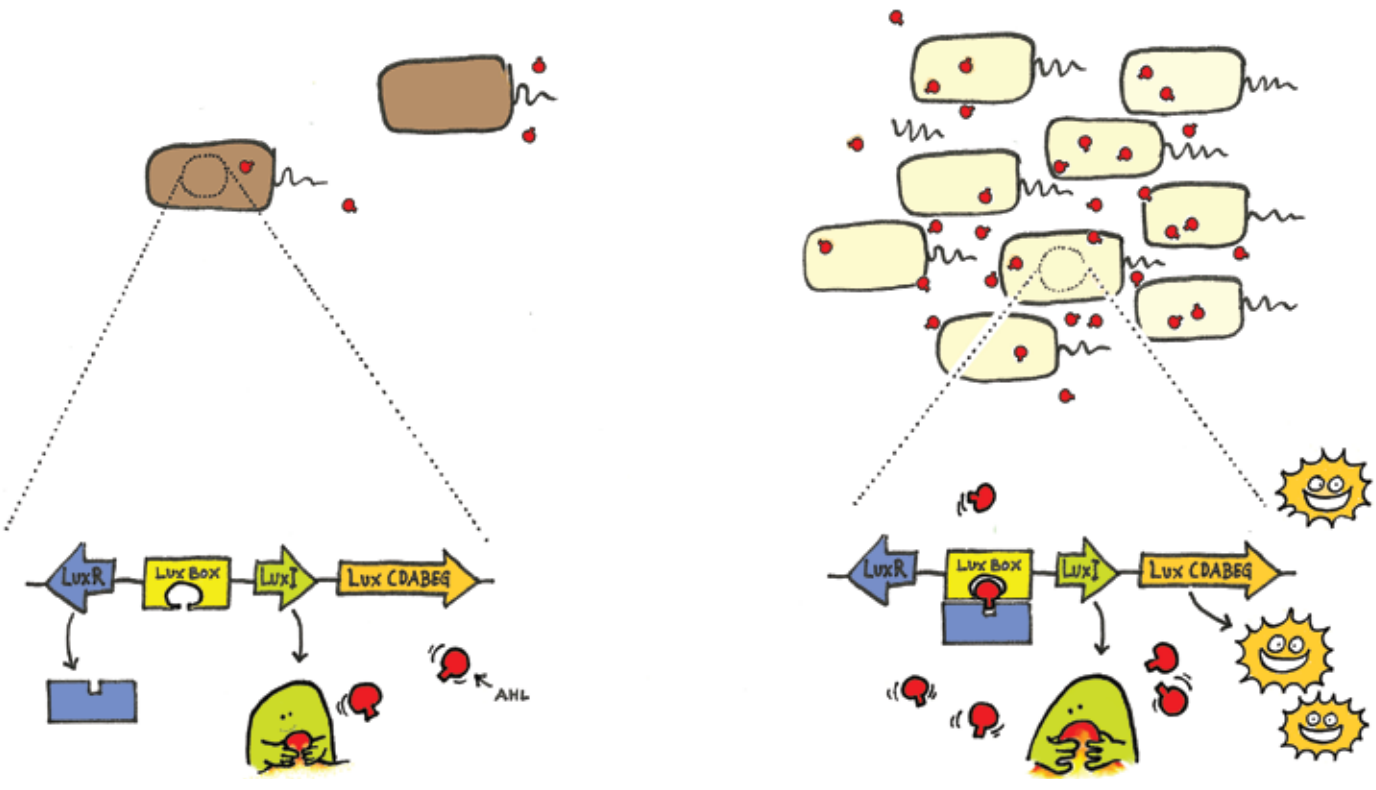
As well as species-specific autoinducers, which allow bacteria to carry out 'secret conversations' with their siblings, bacteria can produce species non-specific autoinducers. Non-specific signals allow the bacteria to assess the numbers and nature of other bacterial species within their community.

Recently, a non-fatty homoserine lactone QS signal, *p*-coumaryl-homoserine lactone (*p*C-HSL) was identified in the photosynthetic bacterium *Rhodopseudomonas palustris* (4). Unlike standard AHL synthesis, where the acyl group is derived from metabolic pools of fatty acids, in *p*C-HSL synthesis the acyl group is derived from the plant metabolite *p*-coumarate. Therefore, it has been proposed that *p*C-HSL acts as both an intraspecies signal to quantify bacterial cell density, and an interkingdom signal between bacterial and plant cells.

Applications of QS research

It is believed that the regulation of pathogenic phenotypes by QS enables bacteria to establish a large population within the host before mounting a concerted attack, thus increasing their chances of overcoming the host immune system. Therefore, it is hoped that the manipulation of QS systems may provide a novel approach for the treatment of bacterial infections. For example, *P. aeruginosa*, which forms thick biofilms in the lungs of individuals with cystic fibrosis, employs QS to control multiple pathogenic phenotypes including the production of virulence factors and biofilm formation. When present as a biofilm, the *P. aeruginosa* cells are highly resistant to many antibiotics, making treatment difficult. If *P. aeruginosa* QS could be disrupted, not only could virulence factor production be prevented, leading to a reduction in lung tissue damage, but biofilm formation would also be inhibited, rendering the cells more susceptible to standard antibiotic treatment.

Drugs that disrupt QS would therefore not kill bacteria like traditional antibiotics but would rather inhibit their pathogenic behaviour. In theory, this would allow enough time for the host immune system or an antibiotic to clear the infection. Therefore, although yet untested, it is hoped that there would



Model of the LuxIR mediated control of bioluminescence in *Vibrio fischeri*. At low cell density (i.e. cells growing in sea water), LuxI produces an acyl homoserine lactone signalling molecule (AHL) which is freely diffusable. In the absence of sufficient AHL concentrations, LuxR does not bind DNA and hence does not activate expression of the luciferase operon (*luxICDABEG*). In contrast, when cells are growing in the confines of the squid's light organ, AHL levels increase with the increasing cell population and reach a minimum threshold concentration. LuxR can then bind to AHL and the resulting LuxR-AHL complex binds to the specific 'lux box' sequence, activating expression of *luxICDABEG* which in turn leads to bioluminescence. LuxR-AHL mediated induction of *luxI* results in even greater levels of AHL production, termed autoinduction. LuxR-AHL causes a decrease in *luxR* expression, forming a negative feedback loop. Image provided by Caroline Dahl.

Quorum Sensing allows bacteria to coordinate their behaviour enabling them to act as multi-cellular units. For example, pathogenic bacteria can launch a 'stealth attack' on their host by switching on the expression of virulence factors only after a large population size has been reached.

be less selective pressure on the targeted bacteria to develop resistance to a QS-disrupting agent than currently seen with traditional antibiotics. Consequently, the 'shelf-life' of QS-inhibiting therapeutics may be longer than that of traditional antibiotics. Professor Bassler's group are currently screening chemical libraries to find QS inhibitors and are developing mammalian model systems in which to test them.

It must not be forgotten that not all bacteria are 'bad'. Just as QS manipulation could be used to inhibit pathogenic bacteria, it could also be used to promote the activities of 'good' bacteria such as those found within the human intestinal tract.

QS is also interesting from an evolutionary perspective as the use of QS allows bacteria to act as enormous multicellular organs, achieving tasks that would be impossible when acting as individuals. Thus, it has been proposed that the systems which allow eukaryotic cells to function as multicellular organisms, with distinct tissue types that are able to identify 'self' and 'non-self', may have evolved first in bacteria.

Concluding remarks

Although originally considered a quirky, niche topic, it is now clear that Quorum Sensing is absolutely fundamental to bacterial physiology, with far reaching effects in the areas of human health, agriculture and biotechnology.

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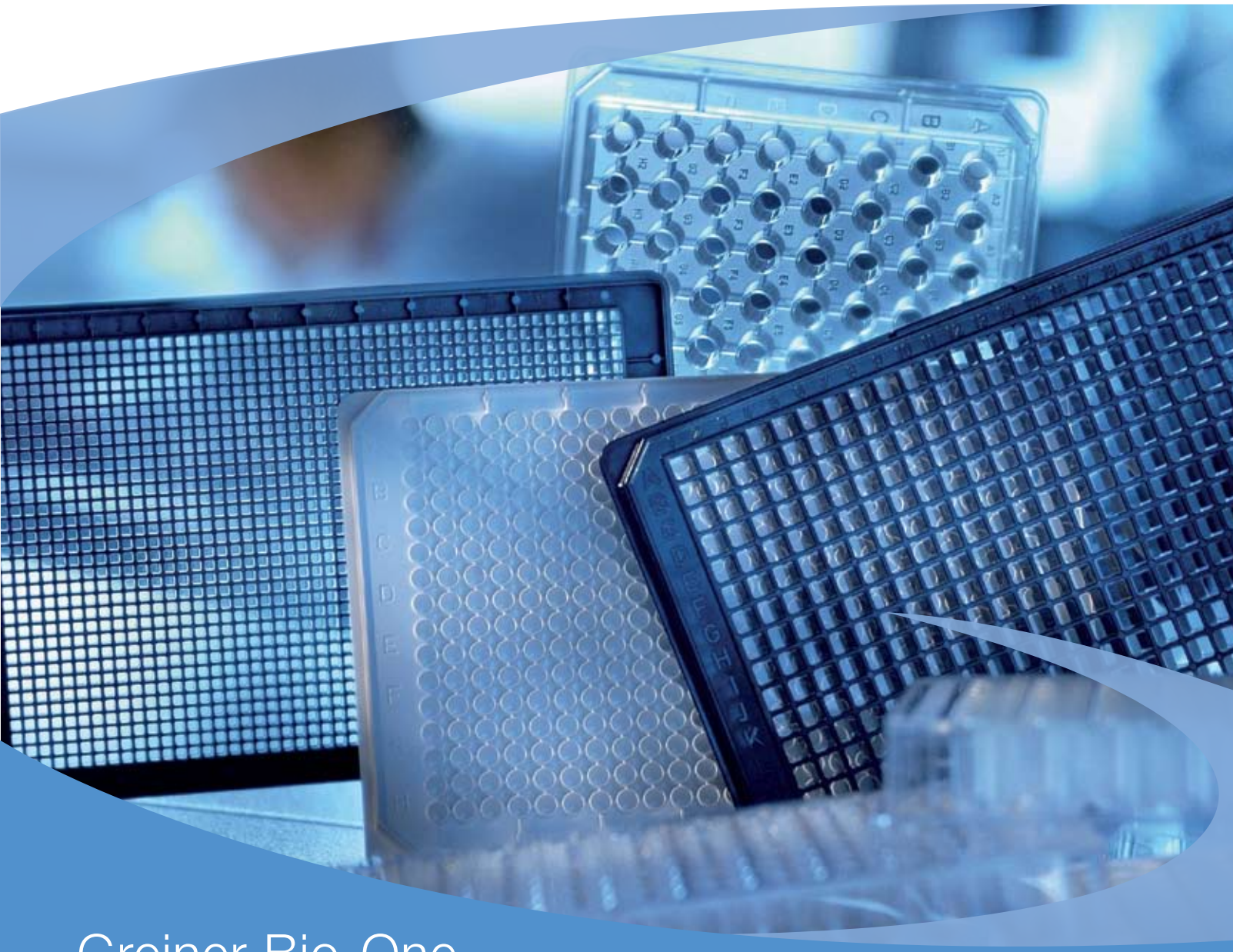

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Tropical Ghosts in Oxford

Penny Sarchet

The *Ghost Forest*, an artwork comprising ten gargantuan uprooted trees, forms an imposing sight in front of the Oxford University Museum of Natural History. Each tree measures over 100 feet and lies on its side on a plinth, its leaves lost, bark weathered, roots exposed. They are a powerful reminder of the drastic rate at which we are removing the 'world's lungs' through deforestation and responses so far have been emotionally charged.

The artist behind the piece is Angela Palmer. Her creative interest in climate change was sparked by a dream in which she visited the most and least polluted places in the world whilst wearing a white suit. She went on to realise this dream, visiting Linfen in China's Shanxi province and Cape Grim at the north western tip of Tasmania, respectively. At both these places she took photographs, video footage, air and water samples, and these were displayed at her exhibition *Breathing In* at the Wellcome Collection this autumn. Whilst developing this project, she began to think about trees, having heard that every four seconds a football pitch-sized area of tropical rainforest is destroyed. In the tamed and drizzly climes of Britain, this devastation seems a very long way away. "You just put it out of your mind and you don't want to confront it", explains Angela.

The *Ghost Forest*, however, brings us face to face with the dead victims of deforestation, making the reality of what we are doing to our remaining tropical wildernesses hard to ignore. It seems to be working. "People want to know more about the trees and they want to know what they can do." Angela is very pleased with this reaction, but as an artist, she stresses that for her any response is valid. "Some people see the trees for their beauty alone and aren't touched by their environmental message. One person was really disturbed by it and didn't want to see the trees at all, she didn't want to feel sad or concerned or experience that confrontation." Angela has found, though, that most people have welcomed the message carried by her trees. "The word that most people use is *awe*. It's an overly-used word but it's what strikes people." Some visitors have cried. "People feel a bit overwhelmed by seeing deforestation. It's really very emotional. I feel very touched by that, it's quite a profound feeling."

A tree trunk completes its long journey from the Ghanaian rainforest to Oxford.

> "I want to see it used as an open air laboratory."

The thoughts and responses of visitors to the *Ghost Forest* have been collected and recorded in a series of interviews by Bronwyn Tarr, a Rhodes Scholar at the Oxford University Environmental Change Institute. Bronwyn is not the only Oxford researcher who has been using the trees since they arrived here in July. Whilst they are here, scientists from departments such as Archaeology and Plant Sciences will be looking at the carbon-dated ages of the trees, their ecology, and their medicinal properties. Robin Dunbar, Professor of Evolutionary Anthropology and Director of the Institute of Cognitive and Evolutionary Anthropology, is interested in looking at human neurological responses to the trees. "I want to see it used as an open air laboratory!" says Angela. She also plans to work visually with the Ruskin School of Drawing and Fine Art, her *alma mater*. "I see it evolving, too" she explains. "It's a good opportunity the year that it's here for people to come up with their own ideas and thoughts."

The *Ghost Forest* trees are a mixture of naturally fallen and sustainably logged trees from Ghana, which although 3000 miles away, is the closest rainforest to the UK. After losing 90% of its primary rainforest over the last 50 years, it now enforces strict regulations, becoming the first African country to join the European Union's effort to outlaw illegal logging. The cooperation and advice of Ghanaian authorities, forest managers and botanists were crucial in bringing the project to fruition. Angela describes the transport of these huge creatures out of the jungle as an organisational nightmare. "When I first had the idea to do it and saw that it was logistically impossible, someone told me I really ought to do it in papier-mâché." However, Angela was reluctant to do this. "It would be like a Walt Disney stage set, and it's not the Never-Never Land, this is really happening. I think to make a powerful impact you have to be brutal about it." So Angela persevered, with expert local advice and input from the University of Oxford.

Getting ten trees, each over 100 feet long, through UK customs...

Once Angela had sourced them, there was the matter of getting ten trees, each over 100 feet long, through UK customs. "The most important thing was that there should be no soil on them. We weren't required to spray them, but we sprayed them anyway in Ghana before they came out here, just to make sure. I didn't want a giant spider landing on Boris Johnson's hand

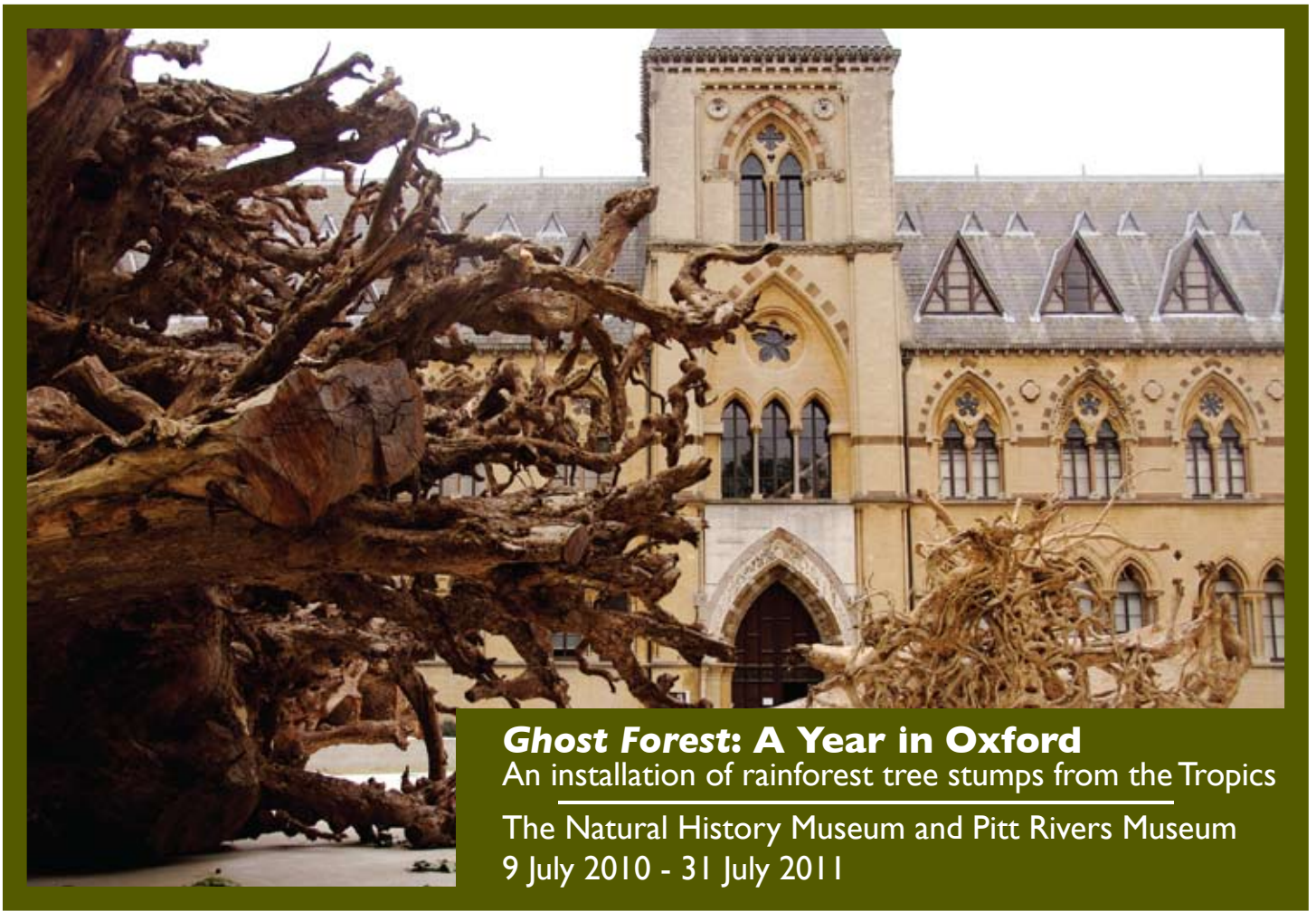
Angela Palmer's *Ghost Forest* installed at the Oxford University Museum of Natural History.

The massive size of the trees impresses all visitors.

in Trafalgar Square!" After London and the UN Climate Change Conference in Copenhagen, Oxford had not been the trees' planned third destination. "They were going to go onto the front quad of UCL but we couldn't get them through the gateway!" Their year in Oxford, however, ties in well with the University's history, forming part of the Museum of Natural History's 150th anniversary celebrations. In 1860, the newly opened museum played host to the famous 'Great Debate' between Thomas Henry Huxley ('Darwin's bulldog') and Samuel Wilberforce, the Bishop of Oxford, over the theory of evolution. The trees' time here also witnesses the transition from the UN International Year of Biodiversity 2010 to the UN International Year of Forests 2011.

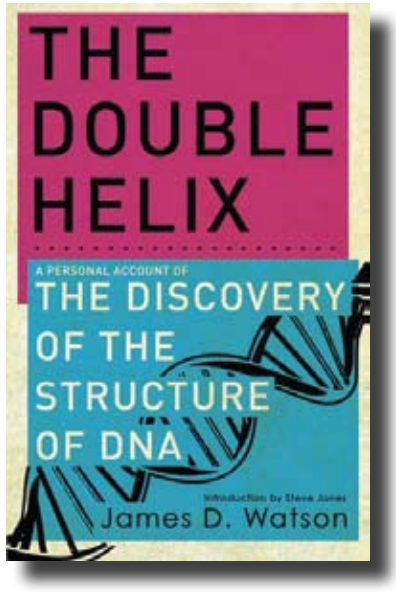
Whilst the *Ghost Forest* is in Oxford, Angela has been appointed Artist in Residence at the Department of Plant Sciences, giving her a chance to pursue all her intended collaborative projects. "It's been amazing how keen scientists are to help and collaborate. A lot of them are interested in seeing their work translated visually and they're very generous with their time." After a project as physically and ambitiously colossal as the *Ghost Forest*, the work of Oxford scientists is in incredibly exciting hands.

Penny Sarchet is a third-year DPhil student in Angela Hay´s laboratory in the Department of Plant Sciences, University of Oxford.



The Double Helix: A Personal Account of the Discovery of the Structure of DNA. By James D. Watson (Reviewed by Jennifer de Beyer)

Published November 2010 by Phoenix (reissue edition), 200 pages, £8.99



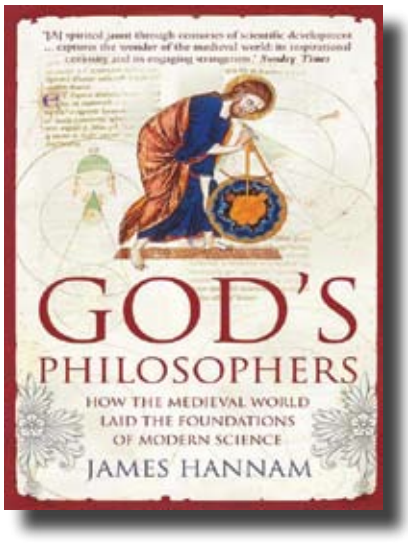
The Double Helix (1968) is an autobiographical account of one of the discoveries that founded molecular biology: the elucidation of the structure of DNA. Dr James Watson's surprisingly candid and personal style set this book apart in 1968 and still does so today.

The author gives an insight into the political and social background, as well as the conversations and chance events, which defined his experience of science in the 1940s and '50s. There is something quite delightful about the gossipy descriptions of his colleagues and rivals, names that we would usually only read in the author list of seminal articles, but are here referred to by first names and nicknames. A self-deprecating tone dominates the early chapters, as Dr Watson describes the start of his career. His journey is an inspiration, emphasising the importance of following opportunities as they present themselves and being open to inspiration from all sources. His descriptions of the collaboration between himself and the late Prof. Francis Crick are also intriguing, with conversations, arguments and the charged emotion of shared discovery laid bare.

Out of the deluge of excellent popular science texts published every year, I hope to have convinced you to reach back for an old favourite and enjoy the drama and suspense of a well-known story. In my opinion, this book is well worth reading, both for the gravitas of the discovery described and the unique style in which it is presented.

God's Philosophers: How the Medieval World Laid the Foundations of Modern Science. By James Hannam (Reviewed by Leopold Kong)

Published August 2009 by Icon Books, 320 pages, £9.99

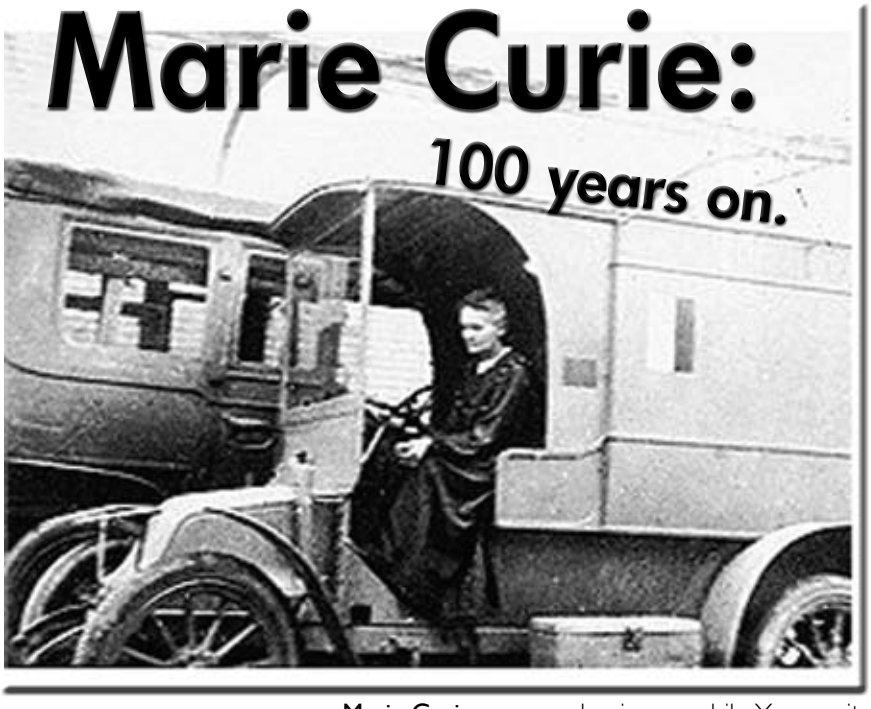


I learnt at school that the Greeks and Romans were exceptionally innovative with their logic and calculating machines. The European Middle Ages, on the other hand, were considered backward and barbaric. Progress stalled until the Renaissance, which marked a wondrous victory of rationality over superstition and was filled with genius personalities. Christopher Columbus was a brave and intelligent explorer who had to convince the very flat-thinking Spanish royalty that the Earth was round. The ideas of Newton, Kepler and Galileo sprung magically from falling apples and swinging pendulums. Many of us do not question these ideas. In *God's Philosophers* however, James Hannam argues that these textbook stories are just myths: the motivations of the ancients were far from rational, the Middle Ages showed great continuity in technical and philosophical innovation, and the Renaissance was characterised more by pretty art and Platonic mysticism than rationality.

Hannam, who trained as a physicist before going into History of Science, writes very clearly and explains complicated concepts in a simple manner. He marshals many lines of historical evidence and his arguments are convincing. For example, he traces the development of the mean speed theorem whose generalisation led to integral calculus. Far from spontaneously emerging from the brains of Newton and Galileo, questions involving average speeds and distances were posed during the Middle Ages. The theorem was explicitly stated by medieval philosophers at Oxford and later proven with geometry by a French cleric centuries before Galileo. Despite this, I think that some of Hannam's demonstrations are oversimplified and forced, potentially causing the reader to question the validity of his hypothesis. One main problem, in my opinion, is that while there were many mathematical and mechanical discoveries during the Middle Ages, it was generally an uncertain time filled with political instability and ignorance of ancient knowledge. European philosophers around 1000 AD knew so little geometry that cutting-edge mathematics involved rationalising why the angles of a triangle added up to 180°!

Overall, this book presents a well-researched and entertaining account of the development of scientific thinking during the Middle Ages. Anyone interested in the history of science would enjoy this work, though supplementary reading is required for a more balanced view.

Marie Curie: 100 years on.



Marie Curie commandeering a mobile X-ray unit.

Alexandra East

Department of Biochemistry

This year marks the 100 year anniversary of Marie Curie's Nobel Prize in Chemistry for her work on the "remarkable element" radium. Marie Curie overcame a great number of obstacles in both her private and professional life to become a pioneering researcher of her day. Her legacy lives on – as recently as 1995, both her and her husband's bodies were moved to the Panthéon in Paris in recognition of their achievements.

Born in 1867 in Warsaw, Maria Skłodowska's early life reflected the difficult political situation in Poland, which had led to her family losing much of their property. After working as a governess to assist her sister financially, Maria moved to Paris in 1891 and enrolled under the French version of her name, Marie, at the Sorbonne to read physics and mathematics.

In 1895, Marie married Pierre Curie, then an instructor at the School of Physics and Chemistry. Shortly afterwards, Pierre earned his doctorate, whereas Marie had yet to choose a topic for her own. Inspiration came from the work of the French physicist Henri Becquerel who had recently discovered that uranium salts emit energy in the form of rays. Marie decided to attempt to isolate other substances which also emitted such rays. In her studies she came across pitchblende, a uranium mineral which emitted rays in a way similar to uranium.

After labour-intensive experimentation, she found that the radioactivity she could detect in pitchblende was much more intense than could result from uranium alone, suggesting the presence of another radioactive element. Pierre Curie was so intrigued by her finding that he decided to drop his own work temporarily and join her. In July 1898, they published a paper announcing the discovery of a new element, which they named polonium after Marie's country of birth. Later that year, the Curies announced the existence of another new element, radium, named after the Latin for "ray", due to its intense radioactivity.

They were awarded a joint Nobel Prize in Physics alongside Henri Becquerel in 1903 "in recognition of the extraordinary services they have rendered by their joint researches on the radiation phenomena". After her husband's sudden death in a street accident in 1906, Marie Curie continued her work on radioactivity and was appointed as the first female professor of the Sorbonne. She was awarded her own Nobel Prize in Chemistry in 1911. During World War I, Marie and her daughter Irène travelled to the front and developed the use of small mobile X-ray units, which could be used to diagnose and treat injuries.

Marie Curie died of aplastic anaemia in 1934, likely caused by excessive exposure to radioactive substances – she was known to carry test-tubes of radioactive material in her pockets, and remark on their pretty glow in the dark.

Marie Curie's remarkable scientific achievements, as well as her character and determination, have inspired generations of scientists and non-scientists alike. Her early work on uranium showed that radioactivity was not the outcome of interactions between molecules but instead must come from the atom itself. Also, the discovery of radium provided scientists with sources of radioactivity with which they could probe the structure of the atom. Marie never patented her technique for extracting radium in order to allow other investigators to also study radioactivity. Radium has since been used to treat cancer – being used in small implants emitting gamma rays which destroy cancerous tissues, although synthetic isotopes are now used more often, as they are safer and easier to handle. Marie's work in this area has provided hope for a huge number of people, given the high prevalence of cancer in the Western world alone. In the United Kingdom, Marie Curie Cancer Care is at the forefront of supporting people affected by cancer. Overall, Marie's efforts in the laboratory and on the battlefield serve as a reminder of her great work and as an inspiration to young scientists. There are also a number of fellowships available in her name, and the European Union's Marie Curie Actions programme has provided funding for over 50,000 of the world's best young researchers since its founding.

STEMNET: Inspiring the scientists of tomorrow

Nicola Platt

Department of Physiology, Anatomy and Genetics

"This is boring." "What's the point?" "It's irrelevant." These are familiar complaints from pupils sitting through hours of dry science and maths lessons. And let's be honest... how many times have you used long division, needed to know the periodic table by heart or designed an experiment using ticker tape since leaving school? It's hardly surprising then that science lessons can seem a world away from students' everyday lives. Yet clearly the science basics learnt at school are the foundation of world leading research and development.

With the advent of new, 'relevant' A-level courses introduced between 1995 and 2005, A-level science entries plummeted. Physics and Mathematics were the worst affected, dropping from nearly 30,000 to 24,000 and from 56,500 to 46,000 entrants respectively. These entries are also not evenly distributed, with over half of science A-level entries originating from just 18% of schools. In concert, over the past decade numerous universities have been forced to close science courses, citing a lack of interest alongside funding issues. How can we ensure that the UK remains among the world leaders in science and technology research? How can we demonstrate the relevance of science to young students? Could you help inspire the next generation of scientists?

Science, Technology, Engineering and Mathematics Network (STEMNET) is a government run programme designed to promote and improve the study of STEM subjects in schools. A key feature of STEMNET is the STEM ambassadors. Ambassadors are volunteers who study or work in a STEM subject area and are passionate about enthusing young students. They span a wide variety of subject areas and career stages, from postgraduate students in basic biochemistry to managing directors of Information Technology companies. The ambassadors' role is to work with teachers to promote the learning and enjoyment of their area of expertise, or STEM subjects in general. This can involve an almost limitless range of activities: helping at a careers evening, running a fun and unusual practical lesson, judging science fairs or organising an after school science club. Recently, for example, Andy, a Materials Science DPhil student and STEM ambassador, ran a class with year 6 students in Didcot who were studying electricity. It involved making batteries using only fruit, a coin and a staple. He said, "The pupils all seemed very engaged with the subject, and it helped to bring the subject of electricity from the abstract into the real and tangible. It also stretched their understanding, and we had some excellent questions and observations during the classes. It was a very electric atmosphere!"

The advantage ambassadors can bring to a school is their expertise. In contrast to science teachers, who often need to spread themselves thinly over the many subjects they are expected to teach, or primary school teachers, many of whom have not studied science in depth since GCSE, the ambassadors know their subject and industry inside out. They also see how the science studied at school has a genuine impact and relevance to the 'real world'. This gives an ambassador much to contribute to a school environment, from imparting additional knowledge or careers advice to sixth form students, to simply helping primary school children understand the significance of their studies. In addition, firing rockets around the school, watching zebrafish embryos or going on farm walks are more exciting than normal lessons and can serve to motivate and enthuse children in the STEM subjects.

Being a STEM ambassador is an easy way scientists can contribute to their local communities and promote public understanding of their research. It also can be of benefit to the ambassadors directly. The questions, challenges and enthusiasm of children can rejuvenate the ambassadors' view of their work and research, and the programme helps young scientists gain a number of transferable skills. Of course, a day out in school is also great fun, and a refreshing change from staring down a microscope all day. It seems fair to say that the STEM ambassador programme is of great benefit for both the scientists of today and of tomorrow.

A STEM ambassador doing an outdoor activity with a group of students in the East Midlands.

Stem Cell Patents – Past, Present and Future

Stephanie Janezic

Department of Physiology, Anatomy and Genetics

Stem cells have the potential to develop into a multitude of cell types during development and may act as internal repair system throughout life. Embryonic and adult stem cells provide a powerful tool to investigate genetic and molecular disease mechanisms and show promise as *in vitro* model system for drug discovery. Perhaps the greatest potential, however, is associated with their use in cell-based therapies to repair or replace damaged tissues as occur in heart disease and spinal cord injuries.

In a fast-moving and promising field such as the stem cell area, intellectual property claims enable scientists to protect their innovations and allow commercialisation of the technology. The most prevalent type of intellectual property protection in the stem cell research area is the patent. Patents offer the patent holder a time-limited monopoly over the patented innovation in exchange for public disclosure of the inventor's technology, allowing the patent holder to exclude others from the use and exploitation of the patented technology.

The United States patent landscape

The United States have historically taken a relatively relaxed stance towards stem cell patent applications and have issued a number of patents covering a range of technologies in the last ten years. These include three patents held by the Wisconsin Alumni Research Foundation (WARF), which were granted to James Thompson for his groundbreaking human embryonic stem (hES) cell research covering the purified preparations of primate ES cells and cell type-specific isolation methods. Other patents granted in the last years cover methods for the use, maintenance and differentiation of hES cells, as well as auxiliary technologies including culture conditions and growth factors.

However, despite the US's more relaxed approach towards patentability issues, research was restricted significantly during the Bush administration due to the prohibition of all federal funding for embryonic stem cell research on the grounds of moral concern. In March 2009, this ban was lifted by an Executive Order of President Barack Obama, which now allows federal funding for research on already-established cells lines.

The European Union patent landscape

The European Union has taken a different stance from the US on the patentability of stem cell technologies and the European field is dominated by concerns about ethical and regulatory implications, which could greatly impact on the commercial viability of emerging technologies.

While patents involving adult stem cells have been granted by the European Patent Office, the patentability of inventions concerning hES cells is more complex and highly controversial. The most important legal provisions giving rise to the controversy of hES cell patent applications are contained in Article 5 and 6 of the European Biotechnology Directive 98/44 EC (see box). Human embryos are included in the definition of the human body in Article 5(1), and totipotent cells, which can differentiate into all cell types required for the formation of the human body, are also covered by the provision. The European Group on Ethics (EGE) therefore held that simple isolation of hES cells by physical separation could not give rise to a patent, while stem cells that are modified *in vitro* for specific applications would represent an "element isolated from the human body" and according to Article 5(2) could therefore be patented.

An important matter arises from the morality clause in Article 6(1) and Article 6(2) of the directive, which prohibits patent applications for technologies that are contrary to morality, in particular when concerning the use of human embryos. This provision raises the difficult issue of requiring a definition of embryos and consequently the starting point of life, which has given rise to a variety of answers from different EU Member

European Biotechnology Directive 98/44 EC:

Article 5

1. The human body, at the various stages of its formation and development, and the simple discovery of one of its elements, including the sequence or partial sequence of a gene, cannot constitute patentable inventions.
2. An element isolated from the human body or otherwise produced by means of a technical process, including the sequence or partial sequence of a gene, may constitute a patentable invention, even if the structure of that element is identical to that of a natural element.

Article 6

1. Inventions shall be considered unpatentable where their commercial exploitation would be contrary to 'ordre public' or morality...
2. On the basis of paragraph 1, the following, in particular, shall be considered unpatentable:

(c) uses of human embryos for industrial or commercial purposes...

States ranging from the fertilisation of an ovum to fourteen days after fertilisation.

The provisions of the European Patent Office are best illustrated by a recent decision of the Enlarged Board of Appeal of the European Patent Office (EPO). In a landmark decision in November 2008, the EPO refused a patent application by the Wisconsin Alumni Research Foundation (WARF) concerning the use of primate embryonic stem cells. The application was held to be contrary to morality for the purpose of Article 6(2) of the European Biotechnology Directive 98/44 EC, stating that European patents shall not be granted in respect of inventions, the publication or exploitation of which would be contrary to ordre public (public order) or morality. The application was also held to be excluded under Article 6(1) as an invention involving the use of human embryos for industrial or commercial purposes. The EPO, however, stressed that while it rejected any patent applications that involve the destruction of human embryos, it did not concern the general question of hES cell patentability.

This decision implicates that, while research on hES cells is permitted in the United Kingdom, which compared to other EU countries has a more liberal approach towards stem cell research, scientists are unable to obtain a patent protection for their innovations.

Access to research

The EPO's decision to reject the WARF patent impacts positively on a further ethical issue concerning the restriction of access to research, created by patents through exclusionary practices or expensive licensing fees, which can have a large impact on the research and development of products and processes with significant medical benefit.

In the US, the debate about access to stem cell technology for research has been mainly fuelled by the three WARF patents granted by the US Patent and Trademark Office. These exceptionally broad patents represent one of the strongest patent claims in the stem cell field and encompass all research using primate hES cells irrespective of their source or application. The patents were sharply criticised by two US consumer groups, Consumer Watchdog and the Public Patent Foundation, who requested a patent re-examination in July 2006. Although initially all three patents were rejected, the patents were later granted with narrower claims and WARF announced a substantial easing of its licensing agreements which already improved the situation for researchers. However, in a more recent development in May 2010, one of the WARF patents was rejected by the US Patent and Trademark Office, a decision which was celebrated by the consumer groups as a "major victory for unfettered scientific research". Both groups stressed that while the inventors did deserve acclaim for their research, important scientific accomplishments should not necessarily be patentable.

The solution: induced pluripotent stem cells?

A promising solution to the complex situation of hES cell patent applications are induced pluripotent stem (iPS) cells, which are derived from adult somatic cells rather than from embryonic sources.

The first mouse iPS cells were generated by Shinya Yamanaka of Kyoto University, Japan in 2006 using the four nuclear reprogramming factors Oct-3/4, Sox2, Klf4 and c-Myc. In 2007, Yamanaka's group in Japan and James Thomson's group at the University of Wisconsin succeeded in producing iPS cells from human somatic cells.

However, while Shinya Yamanaka is often considered the inventor of the iPS cell technology, the patent for creating the method was already granted in 2003 to Rudolph Jaenisch of the Whitehead Institute who said in an interview that: "[While Yamanaka] was the first one to do it, we had the idea first". Yamanaka has since established iPS Academia Japan Inc., which licences patents to both non-profit and commercial organisations to ensure quick access to the technology. Another company involved in the iPS field, the Californian firm iPierian, was granted a patent from the UK Intellectual Property Office in January 2010 to generate human iPS cells from human postnatal cells by using a combination of transcription factors that excludes the oncogenic c-Myc.

Since the new iPS technologies avoid the legal difficulties associated with the patentability of hES cells, we can expect exciting future developments in the stem cell field with fewer legal and ethical controversies. This will allow researchers to generate a large range of tissue types, giving hope for possible personalised therapies in the future.

5'WITH... Professor Judy Armitage

Professor Judy Armitage is a bacteriologist whose research focuses on chemosensory pathways and bacterial motility. She has recently become the director of the Oxford Centre for Integrative Systems Biology and been made a member of EMBO (European Molecular Biology Organization).

When did you first decide that you wanted to be a scientist?
I went to a small girls' grammar school in Yorkshire, with little experience of pupils going to university, so had no pressure and no role models. We had one afternoon of career guidance, where we were all told to be teachers or nurses, but while there was no guidance, there was also no one to tell me I couldn't do something if I put my mind to it. After O-levels it was a toss-up between history and science and in the end science won. I then looked down a microscope and almost instantly knew I wanted to be a bacteriologist. I went to UCL – the first pupil to go to a 'southern' university (i.e. south of Leeds) from my school!

If you had not become a scientist, you would be…
Ancestrally, I should be a Pennine hill-farmer's wife – cooking meat and potato pies and chasing chickens out of the kitchen. However, I think I would have enjoyed being an historian. I think good historical research requires a mind that is open and inquiring, and the need to interpret data without bias is similar to that needed for science.

If you are not in the lab you are…
Asleep – or on a plane, or waiting for a plane! To be honest, being a mother, a more than full-time PI, previously a tutor and now director of OCISB has left me with little time for developing hobbies. I look at my garden, which I promise each year will be transformed into that perfect garden and vegetable patch, and despair. I tend to slump and read (history or detectives) and what I really enjoy doing is cooking (I am quite good).

What has been the most important moment of your career so far and have you had any particularly memorable findings?
The most important moment was probably getting a Lister Fellowship back in the early 80s. That gave me the freedom to develop my own research direction. In terms of memorable findings there have probably been two. One was realising that the bacterium I work on has not one, but two chemosensory pathways that don't crosstalk because they are physically separate in the bacterial cell. This has helped change our view of intracellular organisation in bacteria. The second has a long history, which is really satisfying. When I first started working on bacterial motility (back in the early 80s) I did a series of bioenergetic experiments that suggested that the stators of the rotary motor might disengage when the proton gradient was lost. Using single molecule microscopy, I now know that this was right. We can measure the kinetics of assembly and disassembly of the motor with the changes in driving force. This has changed the textbook view of flagellar motors.

What is the best advice you have ever received?
My old history teacher said I could tackle history at some level as a hobby, but science would be harder. Later, Pat Clarke (UCL) told me, as a young postdoc, not to be hoodwinked into believing all bacteria are *E. coli*.

If you were starting your career again, are there things you would do differently?
My husband is also a scientist and we have not worked in the same city since we were postdocs. I think, rather than assuming we would eventually get jobs in the same place, we should have been more proactive in identifying possible places where we might both work.

Do you have a favourite classical experiment?
If you tether a bacterial cell via its flagellum to a glass slide, the cell body rotates instead of the flagellum. This simple experiment by Silverman and Simon in 1974 proved that the flagellar motor rotated and that the switching frequency changed when challenged with changing chemoeffector concentrations. It is still used as a simple measure of response kinetics in chemotactic mutants.

In your opinion, what makes a good scientist?
Persistence and not following the current fashion. I've had a lot of people tell me things can't work as I suggest because it doesn't work like that in *E. coli* – if the experiment is a good one, believe the results. Also, seeing the potential of a new developing technology and respecting the interests and approaches of others – you never know when a discussion with a chemist or physicist may add a new perspective to your own work.

How do you imagine biological research will change over the next twenty years?
I could not have foreseen the recent changes in sequencing and single molecular microscopy, so I don't think I'm very good at seeing what the future will bring. I just hope I'll recognise it and grab it as it goes past!

SNAPSHOT Research Image Competition

We are very pleased to announce that this issue's winner of the Snapshot scientific image competition is Jessica Tait, a third-year biological sciences undergraduate.

She submitted the impressive photo of a sacoglossan, *Elysia grandifolia*, featured on the front cover. The photo was taken whilst researching epifauna on the prop roots of mangroves on Hoga Island in the Wakatobi Marine Park, Sulawesi, Indonesia.

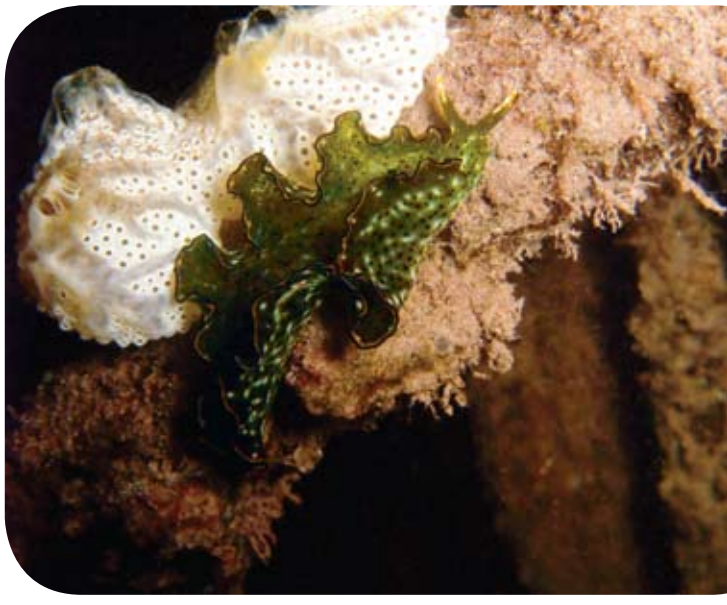
In recognition of her contribution, she will receive a £50 book voucher, kindly provided by our sponsor Oxford University Press.

We hope she will enjoy her reading!

OXFORD UNIVERSITY PRESS

Sacoglossans, commonly known as sap-sucking slugs, are small marine opisthobranch gastropods that belong to the clade Heterobranchia. Sacoglossans live by ingesting the internal contents of green algae, hence the name 'sap-sucking'. Many sacoglossans are green in colour because they sequester living chloroplasts from the algae they eat and utilise them within their own tissues. This very unusual phenomenon, known as kleptoplasty, earns them the title of 'solar-powered sea slugs', and makes them unique among animals.

Jessica was looking for zonation of the root epifauna, both horizontal (as distance from land increased) and vertical (as height above sea level increased), to see if and how shore height or tidal immersion times affected the epifaunal communities. She says the sacoglossan was a chance spotting and was the only one she saw actually on a prop-root surface during the entire trip.



The study showed that diversity increases with distance from land (and thus increased immersion time) as filter feeders and organisms less tolerant to air exposure become more abundant. Up near the shore line there are only brown filamentous algae covering all the roots, with some mud whelks and the occasional grapsid crabs. No sponges really occur above mean tide level, and not many bivalves such as oysters and mussels occur above mean high water. Jessica was also comparing her data with an impacted and pristine mangrove to see how human activity, primarily wood harvesting, would affect the epifaunal communities. She wanted to investigate whether these communities could decline if wood harvesting continues. Collecting this kind of data in the mangroves is quite difficult - at first Jessica planned to work on foot at low tide, but after one exhausting day of trekking through the sediment she decided to collect her data snorkelling at high tide. She used a good supply of waterproof notebooks to record her data and a camera with a waterproof case in order to photograph species she did not recognise so that she could identify them later.

Next year Jessica hopes to do an MSc in London and then hopefully go on to become an Environmental Consultant.

Snapshot Trinity 2011: how to enter...

Do you have an image from, or inspired by your research? Why not enter it in Snapshot?
We are now accepting entries for pictures to be featured in Phenotype Trinity 2011. To enter, send pictures to oubs@bioch.ox.ac.uk with a brief description (maximum 100 words). Please get permission from your supervisor before sending any images. There is no limit to the number of entries per person. The deadline for the competition is 25 February 2011.

crossword

Test your knowledge of the ribosome with this term's *Phenotype* crossword!

Enter the competition by sending your answers to oubs@bioch.ox.ac.uk or leave a paper copy in a sealed envelope in the OUBS pigeonhole at the New Biochemistry reception. Entries received by 25 February 2011 will be entered into the prize draw.

The winner will receive their choice of a recently released Wiley-Blackwell textbook available on the Wiley-Blackwell website.

Congratulations to Roland Ivanyi-Nagy from the Molecular Parasitology Group, WIMM who won the Michaelmas '10 crossword competition.

WILEY-BLACKWELL

OUBS

Across

1. Name given to the UAG codon after Harris Bernstein's last name.
5. The section of an mRNA molecule that binds a small target such as a metabolite, and in response affects the expression of the gene.
9. Trinucleotide sequence that corresponds to an amino acid.
10. Region of the bacterial cell where the ribosomes are located.
14. The stage of protein biosynthesis mediated by the ribosome.
15. Process sometimes called 'charging' the tRNA with the amino acid.
16. The type of RNA that results from the transcription of a DNA template.

Down

2. The 2009 Chemistry Nobel Prize was awarded for studies of the structure and function of the...
3. Eukaryotic organelle containing 70S ribosomes.
4. Female Nobel Prize laureate for Chemistry in 2009.
6. Antibiotic that acts by inhibiting translation.
7. One of the 2009 Chemistry Nobel Prize laureates who gave a talk in Oxford in Trinity 2010 invited by OUBS.
8. Russian-born theoretical physicist who postulated that a three-letter code must be employed to encode the 20 standard amino acids used by living cells to encode proteins.
11. The AUG start codon encodes for this amino acid.
12. In these organisms, protein biosynthesis occurs across the membrane of the endoplasmic reticulum.
13. The nationality of George Emil Palade, the cell biologist who first observed ribosomes in the mid-1950s.