PHENOTYPE

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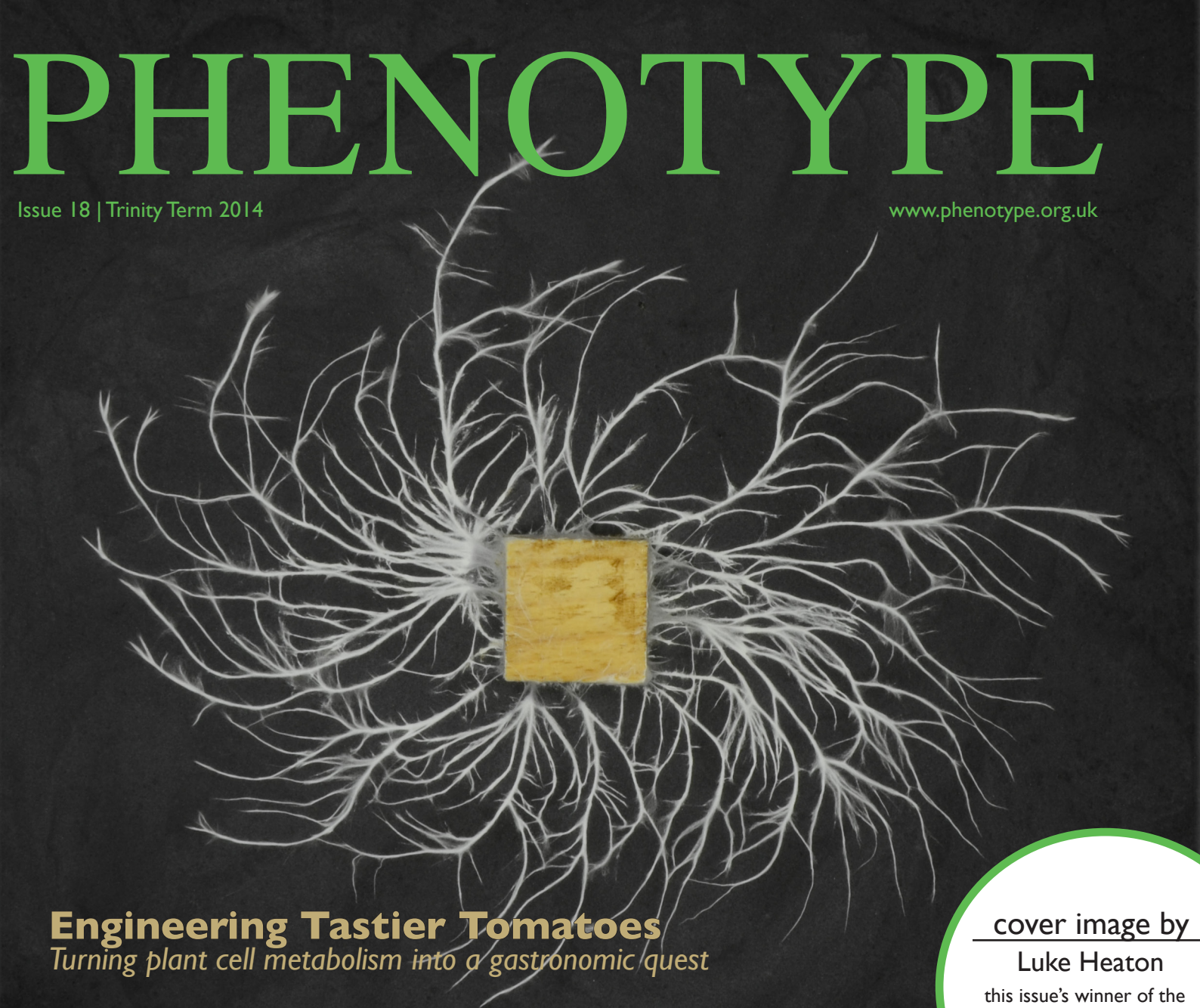

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EDITORIAL

Welcome to the eighteenth issue of *Phenotype*! Again we have another issue blooming with exciting articles contributed by PIs, research staff and students from across the University. But for the first time we bring you a themed issue, showcasing the wonderful world of plant science.

Tired of tasteless supermarket fruit and veg? Dr Lee Sweetlove from the Department of Plant Sciences thinks he's found the key to a tastier tom. Read his terrific tale of transporters and tonoplasts in our featured PI article on page 6.

Lee isn't the only one with a mind for improving crops. In our other features, Sofia Hauck shares the story of the huge diversity in rice cultivars and how we could make use of their ability to survive flooding, while Biochemistry's Prof Simon Newstead sheds light on a dual-action nitrate sensor and transporter with the aim of enhancing uptake. Richard Wheeler's beautiful representation of the plant 'tree of life' educates us on the evolution of this amazing domain of life, which you can see "pressed for time" in herbaria as described by Oxford Herbaria's curator, Dr Stephen Harris.

But plants are more than just food and beauty, as our guests Dr Ross Cloney and Kelvin Chan from Oxbridge Biotech Roundtable explain in their article on plant compounds in cancer drug discovery. Christopher Hillyar also offers us insight into the subcellular targeted delivery of radioisotopes to treat cancer.

Also in this issue, we highlight the connection between plants and the brain. Matthew Warren helps us understand what impact plant flavonoids in our diet may have for the health of our brain, while Dr Ruth Faram advocates that more Alzheimer's research be done using plants.

Interested in more info on careers in science? We feature an interview with Prof Jane Langdale from the Department of Plant Sciences, in which she chats about her path through the field of genetics, as well as Dom Icely's take on being a patent attorney in our Science and Society section. Bethany Palumbo reveals what went on behind closed doors in the Oxford University Museum of Natural History during its refurbishment, and Lydia Le Page invites us to have some Sense About Science (or at least to get help from people who do!).

And if you're interested in DNA repair pathways and their connection to cancer, later this term OUBS will be hosting Prof Alessandro Sartori, Assistant Professor of Molecular Oncology at the University of Zurich Institute of Molecular Cancer Research. Get a preview of his research from Evelyn Tzika on page 5.

Congratulations to Dr Luke Heaton, the winner of last issue's SNAPSHOT competition with his striking photograph of the saprophytic fungus *Phanerochaete velutina*. Further details of his research and budding career can be found on page 31.

Hopefully our congregation of cryptic crossword solvers has come out of hibernation after our last issue's crossword appeared to stump them (answers on page 32!). Have a go at this issue's plant science cryptic crossword by our resident cryptographer *Fish* found on the back cover. Most importantly, if you figure it all out, don't forget to send us your answers for a chance to win one of the excellent Wiley-Blackwell textbooks reviewed on pages 28-29 of this issue!

If you are interested in science communication, writing and publishing, why not join us on the *Phenotype* team? Contact us at **oubs@bioch.ox.ac.uk**! We are always looking for writers, editors and designers to help us with the next issue. Or why not help with getting *Phenotype* out to our audience by assisting with our sponsorship, distribution or social media presence? Being on the *Phenotype* team is a great experience, so I unreservedly recommend getting involved!

Finally, thank you to our amazing and creative *Phenotype* team of post-docs and students who have put this issue together. Their collective hard work and enthusiasm is evident on every page.

Joel Beevers
Editor

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OUBS SEMINARS

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

Featured Seminar

Monday 16 June

Prof Alessandro Sartori, University of Zurich Institute of Molecular Cancer Research, Zurich, Switzerland

For a full list of the Monday seminars, please check the OUBS website: http://www.bioch.ox.ac.uk/oubs/

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OUBS Featured Seminar: Prof Alessandro Sartori

by Evelyn Tzika

This term, the Oxford University Biochemical Society (OUBS) brings you Prof Alessandro Sartori, Assistant Professor of Molecular Oncology at the University of Zurich Institute of Molecular Cancer Research (IMCR).

Prof Sartori obtained his PhD in Biochemistry from the University of Zurich, focussing on the functional role of uracil-DNA glycosylase and how it interacts with other molecules. From here, Prof Sartori developed a research interest in DNA repair pathways. He then carried out post-doctoral research at the University of Cambridge, before returning to the University of Zurich as a Senior Scientist in the IMCR, funded by the University and the Swiss National Science Foundation. During his time in the field, Prof Sartori has authored a number of significant publications and in 2008 received the Dr Ernst Th Jucker Award for his outstanding research and pioneering approaches to the field of DNA repair and cancer therapy.

One of his major areas of research is the role of DNA Helicase Q (HELQ) in mammalian cells. The repair of interstrand crosslinks (ICLs), a type of DNA damage, is normally achieved by the cooperation of the intra-S-phase checkpoint and the Fanconi anaemia pathway. Prof Sartori and colleagues showed that HELQ directly interacts with the BCDX2 complex, which is required for homologous DNA repair, and functions in parallel with the Fanconi anaemia pathway to promote efficient recombination at damaged replication forks. This revealed a critical role of HELQ in replication-coupled DNA repair, germ cell maintenance and tumour suppression in mammals (1).

Currently, Prof Sartori's group largely focusses on the molecules and factors that take part in the repair of DNA double-strand breaks (DSBs), one of the main causes of DNA damage that can lead to cancer. There are two different evolutionarily conserved mechanisms that enable the repair of DSBs: homologous recombination and non–homologous end joining. The Ct-interacting protein (CtIP) is known to interact with both the C-terminal binding protein (CtBP), a transcriptional co-repressor, and the product of the tumour suppressor gene *BRCA1* to control DNA repair and cell cycle checkpoint control. Prof Sartori and his team found that CtIP also cooperates with the MRN complex to repair DSBs by DNA end resection, a process of making single stranded DNA ends as a required step towards homologous recombination (2). Furthermore, CtIP-dependent DNA end resection may actively suppress non-homologous end joining, the second major DSB repair pathway in human cells, which simply rejoins DSB ends and can introduce mutations. The repair of DSBs, and therefore the activity of CtIP, is hence crucial for the maintenance of genomic stability.

Previous work has shown that the initial checkpoint response in S and G2 phase is dependent on the serine/threonine protein kinase CHK1. Prof Sartori's team has shown that this pathway operates prior to DNA end resection, thus preventing cells with damaged DNA from replicating their DNA or entering mitosis (Figure 1). After homologous recombination is initiated by CtIP, sustained checkpoint signalling then ensures sufficient time for DNA repair by preventing mitotic entry until the repair process is complete (3). In addition to its role in G2/M checkpoint maintenance, they also discovered that CtIP is required for the S-phase checkpoint.

The research of Prof Sartori and his group on DNA repair and cancer therapy has great implications in the field that could lead to a revolutionary approach in the design of future treatments.

References

1. Adelman CA, *et al.* (2013) HELQ promotes RAD51 paralogue-dependent repair to avert germ cell loss and tumorigenesis. *Nature* 502(7471):381-384.

2. Eid W, *et al.* (2010) DNA end resection by CtIP and exonuclease 1 prevents genomic instability. *EMBO* Rep 11(12):962-968.

3. Kousholt AN, *et al.* (2012) CtIP-dependent DNA resection is required for DNA damage checkpoint maintenance but not initiation. *J Cell Biol* 197(7):869-876.

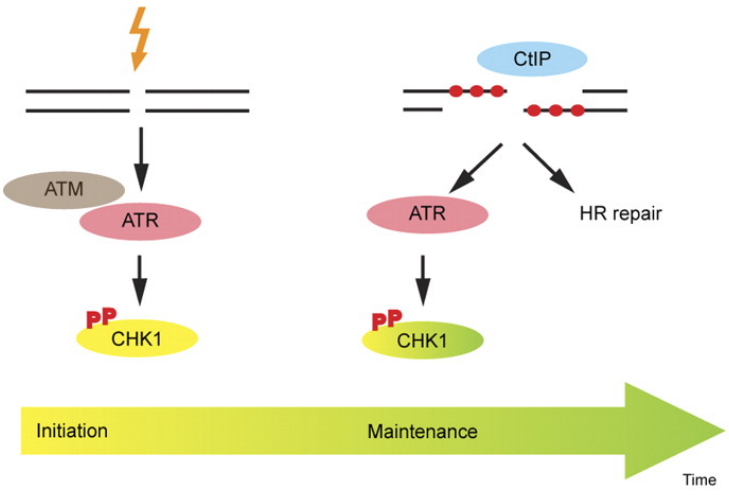


Figure 1: Following DNA damage, cell cycle check points are activated independently of DNA end resection by ATM, ATR and CHK1 protein kinases. This is followed by resection-dependent maintenance of S and G2 phase checkpoints, which is initiated by CtIP. Figure ©Kousholt AN, *et al.*, 2012. Originally published in JCB. doi:10.1083/jcb.201111065.

Engineering tastier tomatoes

by
Dr Lee
Sweetlove

The moment you start thinking 'things ain't what they used to be', you can be pretty sure that you're not as young as you once were. It may be breaking every rule in the book to start with not one, but two clichés, but there is comfort and truth in such sayings. And when it comes to tomatoes, one of the plant species that my group works on, the truth is palpable. Tomatoes most certainly do not taste like they used to. Much commercial tomato production is disappointing when it comes to flavour. Tomatoes should be bursting with rich, complex flavour, an intermingling of sugars and acids and a waft of volatiles that ignite the senses. Bite into a supermarket tomato and you are apt to be disappointed. The fruit will be insipid, watery and often hard. Close your eyes and you could be eating an apple. And yet, tomatoes are the world's most consumed fruit and a billion dollar crop globally.

The reasons behind this state of affairs are familiar: a food supply chain that values quantity over quality and a consumer market that is driven by value for money over flavour. Farmers find themselves in the unenviable position of having to produce ever-increasing yields per hectare in order to meet their contracted price point, and supply a product in which uniformity of size and appearance is valued more than flavour and nutrition. Seed companies have responded to these market demands by selecting for high-yielding tomato varieties, yield being the total weight of fruit produced per plant. And the easiest way to increase fruit weight? Fill it up with water. Herein lies the problem: add water – dilute the taste. The issue also goes beyond mere palatability. Fruits such as tomatoes are rich in antioxidants and vitamins – better tasting fruits are likely to lead to increased consumption of these.

My research group focuses on plant metabolic networks, and flavour is a product of metabolism. In the case of tomatoes, flavour develops during the ripening process. The fully-grown green fruit undergoes a visible metabolic transformation. Chlorophyll is broken down and red carotenoid pigments are synthesised. The fruit softens due to cell wall degradation and, crucially, metabolites accumulate. Of particular importance for flavour are the hexose sugars, glucose and fructose, and acidic metabolites such as citrate, aspartate and glutamate (Figure 1). The correct balance between sugars and acids is what characterises a flavourful tomato. As the acidic compounds are synthesised from Hans Krebs's famous biochemical cycle (Figure 1) – a part of metabolism we are experts in – we have focused our efforts on figuring out how to increase the accumulation of acidic metabolites, so boosting fruit flavour.

The genetics of flavour

Tomatoes are easy to genetically modify using the infective *Agrobacterium tumefaciens* to transfer gene constructs into the tomato genome. This means we can use RNA interference or antisense RNA to suppress specific genes or introduce new gene copies, either from tomato or other species. We can therefore tweak the genome, increase or decrease the expression of genes encoding the relevant enzymes and achieve greater accumulation of flavour metabolites. But which are the relevant enzymes? The Krebs cycle may only consist of eight enzymes, but it is embedded in a wider network of reactions without which it cannot function. In fact, metabolism is a 'small-world' network and the high degree of connectivity means that you consider a particular subset of reactions in isolation at your peril. Thus, the great challenge facing the metabolic engineer is to identify which of the hundreds of potentially relevant reactions to target for the desired outcome, whilst simultaneously causing minimal perturbation across the rest of the network. We use two approaches to address this challenge – genetics and computational modelling. I will outline the genetic approach in this article.

Our starting point was a population consisting of a series of lines of the cultivated tomato species *Solanum lycopersicum*, that have been partially hybridised with the wild, green-fruited species, *Solanum pennellii*, so as to introduce a single chromosome fragment per line: a process known as introgression (1). Phenotypic changes as a result of this genetic variation include variations in metabolism. We identified a number of lines from the population producing fruit with higher levels of

Image by Óscar Cordero Llana.

acidic flavour metabolites, such as citrate, aspartate and glutamate. We then asked a simple question: what were the metabolic changes in these lines associated with the altered metabolite profile? After extensive screening of gene expression, enzyme activity and metabolic flux, we could only find one consistent change in the system that correlated with increased citrate content: a decrease in the cytosolic isoform of the enzyme aconitase. This makes sense. The reaction catalysed by aconitase converts citrate to isocitrate, so by decreasing the amount of this enzyme one can expect citrate to accumulate. We proved aconitase was responsible by expressing an antisense construct for the aconitase gene. Aconitase activity decreased by 30% in these transgenic plants and this led to a proportional increase in citrate levels in ripe fruit (2). Targeting this enzyme may seem obvious, but there are multiple reactions and metabolic exchanges that converge on citrate and numerous enzymes, besides aconitase, have been proposed to control citrate accumulation.

Storage is more important than synthesis

Nevertheless, the link between aconitase activity and citrate content was straightforward. What was harder to explain was that in lines accumulating up to two-fold more aspartate and glutamate, there were no detectable changes in metabolic capacity in any relevant parts of the metabolic network. Transcripts encoding enzymes involved in aspartate and glutamate synthesis were unaltered, as were enzyme activities and flux through central metabolism and the Krebs cycle giving rise to these two metabolites. How could aspartate and glutamate accumulate at faster rates while the metabolic pathways generating them were unaltered in capacity or flux? A clue was revealed from analysis of metabolic network fluxes in cell suspension cultures of a different plant: *Arabidopsis thaliana*.

In this system, the rate at which metabolites were 'bled off' the Krebs cycle for storage was tiny in comparison to flux round the cycle itself (3). In other words, you could increase the rate of storage of these metabolites and the effect on the Krebs cycle would be negligible. The implication is that the Krebs cycle enzymes are unlikely to significantly control the accumulation rate of derived metabolites like aspartate and glutamate.

Our metabolic bias had been obscuring the answer. Mature tomato fruit cells are dominated by a huge central vacuole which occupies up to 95% of cell volume. For metabolites that reach concentrations of tens, or even hundreds, of millimolar (mM) on a bulk-tissue basis – which, in tomato, includes sugars, citrate, aspartate and malate – most of the compound must be localised within the vacuole. Sequestration into the vacuole is mediated by transporter proteins that transfer metabolites from the cytosol, across the vacuole membrane (tonoplast) and into the vacuole lumen. Could these transporters be limiting the rate of vacuolar accumulation of aspartate and glutamate? This turned out to be a question easier to ask than to answer – almost nothing was known about amino acid transport across the tonoplast in tomato or any other plant species. Plant genomes contain huge families of genes encoding amino acid transporters – over 70 in *Arabidopsis* – very few of which have been characterised. Those which have been so far all localise to the plasma membrane, not the tonoplast. We were faced with the proverbial needle in a haystack.

To try to pick the tonoplast aspartate and glutamate transporters out of the haystack, we devised a proteomic strategy. We reasoned that the rapid increase in accumulation of these amino acids during ripening (Figure 1) is likely to be

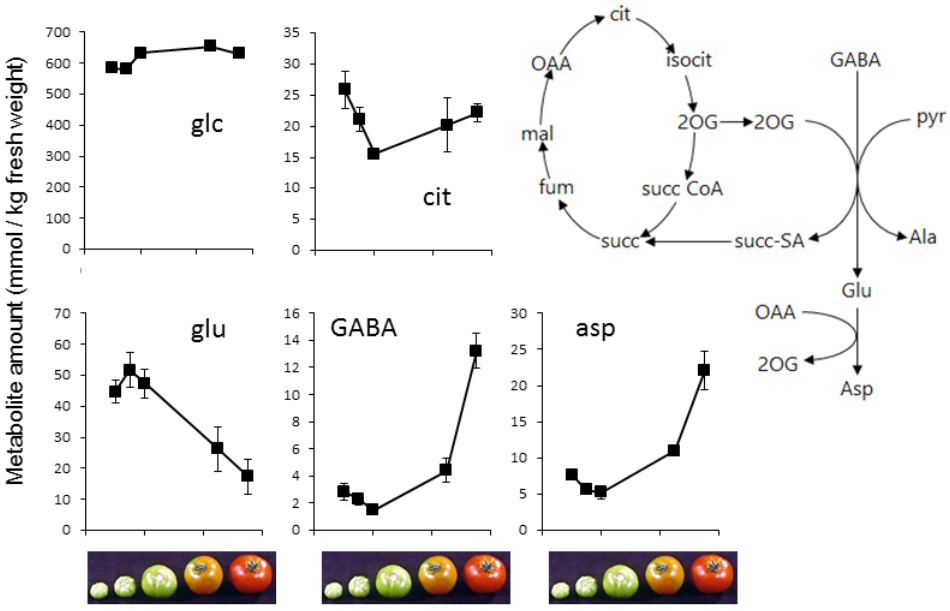


Figure 1: Changes in key metabolites during fruit development. Metabolites were quantified in pericarp tissue of *Solanum lycopersicum* fruit at different stages of development, as indicated in the photograph. Values are the mean of 6 fruits from independent plants ± SEM. The metabolic interconnections between these metabolites are show in the pathway diagram on the right. Abbreviations: 2OG, 2-oxoglutarate; ala, alanine; asp, aspartate; cit, citrate; fum, fumarate; GABA, γ-amino-butyric acid; glc, glucose; glu, glutamate; isocit, isocitrate; mal, malate; OAA, oxaloacetate; pyr, pyruvate; succ, succinate; succ CoA, succinyl-coenzyme A.

accompanied by increased presence of the respective tonoplast transporters. We therefore purified tonoplast membranes from fruit before, during and after the increase in aspartate and glutamate, and quantified the integral membrane proteins in the tonoplast samples by mass spectrometry. We identified two candidate proteins that were members of amino acid transporter families and that showed the requisite abundance profile. Of these, the gene for only one fell in a genetic quantitative trait locus for the relevant amino acids. The gene, *CAT9*, belongs to a sub-family of amino acid transporters that predominantly transport cationic amino acids, but some members have been shown to transport anionic amino acids.

The *CAT9* gene was functionally characterised by over-expressing it in tomato. Transport-competent vesicles formed from tonoplast membranes from the transgenic fruit had a significantly greater transport capacity for glutamate in an exchange mode with glutamate (homo-exchange), aspartate and GABA. Transport of the latter was a surprise, because the only other characterised GABA transporter – the plasma membrane localised GAT1 protein – is proton-coupled and does not exchange GABA with other amino acids. The possibility of importing glutamate and aspartate into the vacuole whilst exporting GABA made us examine the changes in the amounts of these amino acids during fruit development (Figure 1). The decline in GABA content that begins early in development is roughly equivalent to the increase in aspartate and glutamate. This suggests the metabolic exchange illustrated in Figure 2. GABA is released from the vacuole and is imported into the mitochondrion, where it is metabolised into aspartate and glutamate. These two amino acids are transported into the cytosol and then into the vacuole, in exchange for more GABA efflux across the CAT9 glutamate/aspartate/GABA exchanger. Having identified this key tonoplast transporter, we could finally test our hypothesis that it may exert considerable control over the accumulation rate of aspartate and glutamate. We quantified both amino acids in transgenic fruit over-expressing CAT9. Both amino acids were increased: aspartate dramatically so, by more than five-fold. This increase is huge considering that aspartate was already the most abundant amino acid in our ripe fruit, reaching a concentration of some 80 mM in the genetically modified fruit. Glutamate was also increased by just under two-fold.

Naturally, we could not resist tasting the genetically modified tomatoes. It is early days: we used a lab-variety, Micro-Tom, for the genetic modification and it is not the tastiest of tomatoes to start with. Nevertheless, everyone who tried the transgenic tomatoes (seeds removed!) could discriminate the fruit over-expressing the transporter gene from wild-type fruit and all perceived an improved flavour. We are currently working with our industrial partner, Syngenta, who part-funded this work, to make similar genetic changes to their commercial tomato varieties using advanced molecular breeding techniques. It may take some time, but maybe one day we will see tomatoes with altered vacuole transporter properties in our supermarkets.

References

1. Eshed Y & Zamir D (1995) An introgression line population of *Lycopersicon pennellii* in the cultivated tomato enables the identification and fine mapping of yield-associated QTL. *Genetics* 141(3):1147-1162.

2. Morgan MJ, *et al.* (2013) Metabolic engineering of tomato fruit organic acid content guided by biochemical analysis of an introgression line. *Plant Physiol* 161(1):397-407.

3. Williams TCR, *et al.* (2008) Metabolic network fluxes in heterotrophic *Arabidopsis* cells: stability of the flux distribution under different oxygenation conditions. *Plant Physiol* 148(2):704-718.

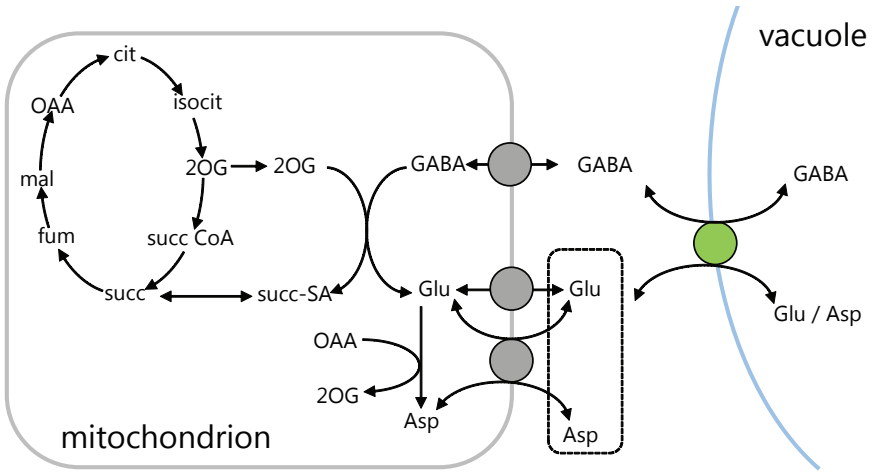


Figure 2: Schematic showing the route for interconversion of vacuolar GABA into glutamate and aspartate. The vacuole membrane is shown in blue and the mitochondrial membrane in grey. Transporters are indicated as circles, with the green-coloured circle representing the CAT9 GABA/glutamate/aspartate transporter. Abbreviations are as for Figure 1.

Dr Lee Sweetlove is a group leader in Plant Sciences.

RESEARCH HIGHLIGHTS

by Alexander Feuerborn

Vance KW & Sansom SN, *et al.* (2014) *EMBO J* 33(4):296-311.

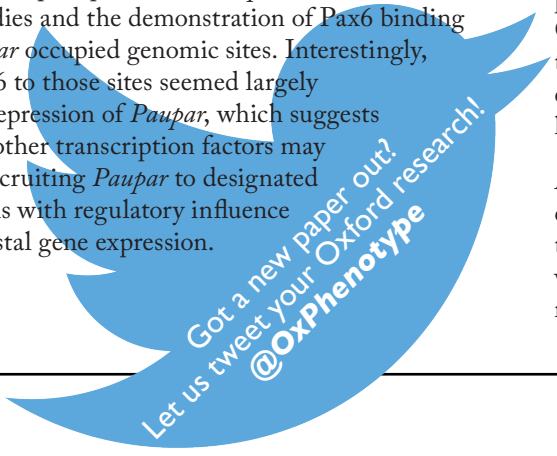
The long non-coding RNA *Paupar* regulates the expression of both local and distal genes

Long non-coding RNAs (lncRNAs) comprise diverse RNAs whose functional relevance remains largely unknown. They can affect transcription of genes in proximity to sites of their own synthesis. Recent studies have now expanded the putative sites of action of lncRNAs, owing to discoveries of their genome-wide interaction with chromatin.

In their joint co-authored paper, Vance & Sansom *et al.* explored the transcriptional function of the lncRNA *Paupar*, which is 8.5 kb upstream of *Pax6* encoding a transcription factor involved in neurogenesis. The authors found that the *Paupar* transcript is mostly chromatin-associated and, at 3.48 kb long, has a relatively high degree of conservation compared to other lncRNAs. Both *Paupar* and *Pax6* display high expression levels in the adult mouse brain and overlapping expression patterns during retinoic-induced neuronal differentiation, indicating that *Paupar* and *Pax6* may coordinate similar biological processes.

The use of microarray-based gene expression profiling to compare the genome-wide influence of *Paupar* and *Pax6* showed that differentially expressed genes commonly affected in cells with knockdown of *Paupar* or *Pax6* are positively correlated and implicated in synaptic functions. This suggests that *Paupar* and *Pax6* may collaborate in coordinating the expression of functionally distinct sets of genes. However, *Paupar* also influenced the expression of numerous genes that were non-responsive to Pax6-repression. The authors thus concluded that *Paupar* influences gene expression in Pax6-dependent and independent manners.

Notably, employing 'CHART-seq' methodology, Vance & Sansom *et al.* found that *Paupar* largely associated with promoters and 5' UTRs of protein-coding genes. *In silico* analysis of these regulatory interaction sites revealed the enrichment of the binding motifs of *Pax6* and other neural transcription factors, indicating that *Paupar* targets genomic regions by physically interacting with distinct transcription factors. This is supported by the RNA-immunoprecipitation of *Paupar* with Pax6-specific antibodies and the demonstration of Pax6 binding to several *Paupar* occupied genomic sites. Interestingly, binding of Pax6 to those sites seemed largely unaffected by repression of *Paupar*, which suggests that Pax6 and other transcription factors may play a role in recruiting *Paupar* to designated genomic regions with regulatory influence on local and distal gene expression.



Loenarz C, *et al.* (2014) *Proc Natl Acad Sci USA* 111(11):4019-4024.

Hydroxylation of the eukaryotic ribosomal decoding center affects translational accuracy

Altering levels of oxygen directly influences gene expression. An example of this is the hydroxylation and subsequent turnover of hypoxia-inducible transcription factors under normoxic conditions.

In this paper, Loenarz *et al.* assessed whether oxygen-dependent modifications of proteins may affect translational processes. Intact protein mass-spectrometry and dot-blot analyses of ribosomal proteins from *Saccharomyces cerevisiae* grown at various oxygen levels identified the ribosomal protein Rps23p as a di-hydroxylated protein under normoxic conditions, but which was mono- or non-hydroxylated under hypoxic or anoxic conditions.

Mutations in *RPS23* have been associated with faulty stop-codon recognition. The authors tested whether hydroxylation of Rps23p could similarly affect translational accuracy. In order to study translational read-through, the authors used a construct consisting of the *Renilla* and Firefly luciferases separated by the in-frame stop-codon from the 'Bypass of stop codon protein 4' gene (*BSC4*). These dual luciferase assays showed that yeast cells grown under normoxic and hypoxic conditions recognised the respective stop codon less accurately than yeast grown anoxically. Interestingly, yeast lacking Tpa1p (*tpa*–) – the homologue of human 2-oxoglutarate and iron-dependent oxygenase domain-containing protein 1 (OGFOD1) – revealed increased stop-codon recognition even when grown under normoxic conditions. Importantly, the effect of Tpa1p deletion on stop-codon read-through is context dependent, as demonstrated by an elevated read-through across a sequence fragment containing the stop codon of another gene. This, combined with the absence of Rps23p-hydroxylation in *tpa*– yeast, suggests that Tpa1p catalyses hydroxylation of Rps23p, thereby affecting translational accuracy in a sequence and context dependent manner.

Co-expression of Tpa1p/OGFOD1 homologues from lower eukaryotes with GST-tagged human RPS23 followed by proteomic tandem-mass-spectrometry suggests that di-hydroxylation of RPS23 is a conserved modification across lower eukaryotes. In contrast, a companion article published in the same journal issue shows that human OGFOD1 only mono-hydroxylates RPS23. This indicates that RPS23 hydroxylation is conserved across eukaryotes but differs between eukaryotes with regard to mono- and di-hydroxylation.

Additional luciferase read-through experiments using stop-codons in clinically relevant contexts, strongly suggest that the first nucleotide following a stop-codon decisively affects whether translational accuracy is reduced or enhanced in response to Rps23p-hydroxylation.

Flavonoids: More than just pretty colours

by Matthew Warren

In modern medical research, the way in which nutrition affects our health is frequently overlooked. Investigations into the effects of the substances that we put into our body every day are often sidelined in favour of the search for the ever-elusive panacea. Nowhere is the importance of nutrition in health and disease more apparent than in the case of flavonoids. The significance of these plant compounds in cardiovascular health has become evident over the last few decades, but recently neuroscientists are beginning to realise that flavonoids could also be invaluable in treating brain disorders, particularly dementia.

Flavonoids are metabolites found in all vascular plants, that is, plants with conducting tissue (xylem and phloem). One of their key roles is to produce the colour of leaves, flowers and fruit, which makes the plant attractive to pollinating and seed dispersing animals. A class of flavonoids called anthocyanins act as pigments that create the vivid colours of blueberries and red cabbage. Other classes interact with anthocyanins in a process called copigmentation to further enhance the colour. It is no coincidence that the flavonoid-rich foods that we are encouraged to consume are among the most vibrant.

Flavonoids are also fundamental in protecting plants from damage by ultraviolet radiation. UV light induces the biosynthesis of flavonoids, which accumulate in the epidermis, the outermost cell layer of the plant. Here they absorb UV light, protecting the plant from potential DNA damage or other impairments caused by excessive UV exposure. These wonder-molecules are also thought to have anti-microbial functions, contribute to pollen development, and may even be involved in the structure of plant tissues. *In vitro* assays have demonstrated that flavonoids are strong antioxidants, although antioxidant effects have yet to be shown in plants. However, it is these antioxidant properties which are most attractive to researchers interested in the possible health benefits of human flavonoid consumption. Researchers have been particularly interested in the effects of flavonoid consumption on the cardiovascular system. The media often report stories about how a daily glass of red wine or a square of dark chocolate confers protection against heart disease. Although these claims may be somewhat overblown, increased flavonoid consumption does seem to be at least slightly protective against cardiovascular disease. This effect may be partly due to the antioxidant properties of flavonoids, for example by preventing the oxidation of low-density lipoprotein, which leads to accumulation of cholesterol in arteries (1).

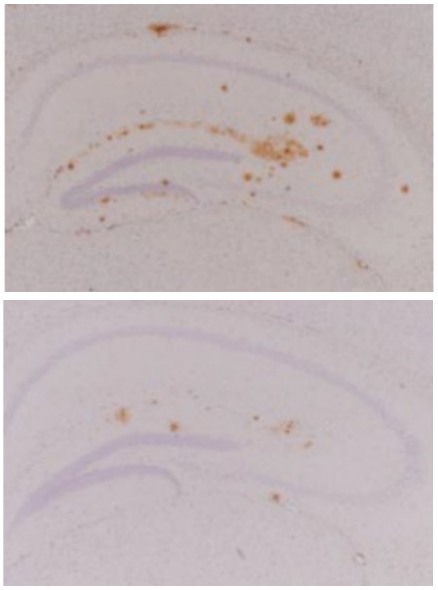


Figure 1: Hippocampal slices showing amyloid plaques in vehicle-treated (top) and nobiletin-treated (bottom) animals. Figure from (2), used with permission.

Neuroscientists are interested in whether these compounds could be used in preventing – or even treating – Alzheimer's disease and other dementias. Although Alzheimer's disease is thought to be multifactorial many researchers believe that oxidative stress plays a major role. Therefore, it may be possible to use flavonoids to scavenge free radicals that contribute to the disorder.

Trials using flavonoids to treat rodent models of Alzheimer's disease have yielded promising results. One of the key features of Alzheimer's disease is elevated brain levels of β-amyloid (Aβ) peptides, which aggregate into amyloid plaques. In rodent models of the disease, the amyloid precursor protein (APP) is overexpressed, producing amyloid plaques and memory impairments. In one study, researchers gave APP-overexpressing mice daily injections of either nobiletin, a flavonoid found in citrus fruit, or a vehicle for four months (2). In memory tasks, mice treated with nobiletin had fewer memory deficits compared to the control group. Moreover, on post-mortem analysis, the nobiletin group showed a 60% decrease in the number of Aβ deposits in the hippocampus, an area of the brain involved in memory formation that is particularly vulnerable to damage in Alzheimer's disease (Figure 1).

Human research has also shown promising results. In a longitudinal study (3), 1367 healthy French adults over 65 answered questionnaires on the frequency with which they consumed various foods and drinks. The researchers determined the approximate quantity of flavonoids consumed by each participant, based on the known flavonoid content of each food. Five years later, all participants were assessed for signs of dementia. Those who had previously had high or medium levels of flavonoid intake were less likely to suffer from dementia than those who had consumed fewer flavonoids.
Findings like these are encouraging. However, it is possible that the protective effects of flavonoids in

the human brain are not due to their antioxidant properties. Unlike in the test tube, in the human body flavonoids are quickly metabolised, and these metabolites are not antioxidants. Furthermore, the levels of flavonoids and their metabolites in the brain tend to be much lower than those of other antioxidants, so it is hard to see how they could be beneficial. How then do flavonoids exert their neuroprotective effects?

There are a number of possibilities, which are not mutually exclusive (Figure 2). In Alzheimer's disease, the level of proinflammatory cytokines is elevated, which is thought to contribute to the pathophysiology of the disease. It is possible that flavonoids act to downregulate the production of these inflammatory molecules, thereby protecting neurons from damage.

Perhaps the most intriguing possibility is that flavonoids actually encourage the generation of new neurons. Neurogenesis occurs throughout the adult lifespan in a small number of brain regions, including part of the hippocampus called the subgranular zone (SGZ) and another area called the subventricular zone (SVZ). It also appears that flavonoids modulate intracellular signalling by the ERK pathway, ultimately increasing expression of brain-derived neurotrophic factor (BDNF), a protein responsible for the development and survival of neurons, which is typically downregulated in Alzheimer's disease.

Additionally, flavonoid consumption can increase blood flow to the brain and encourage the formation of new blood vessels. The endothelial cells that line blood vessels release factors that stimulate the production of new neurons and the net result of these changes will be an increase in neurogenesis. There is increasing evidence from animal studies that this is the case and that flavonoids do encourage neuronal generation. In a recent investigation (4), researchers temporarily reduced blood flow to the brains of mice in order to cause cell death and then treated the mice with either 25 or 50 mg/kg heptamethoxyflavone - another flavonoid from citrus fruit - or vehicle daily for three days. Mice who had received heptamethoxyflavone showed a far greater number of neuronal precursor cells in either the SGZ or SVZ, suggesting that administration of the flavonoid increased the production of new cells in the brain.

The shift of focus away from the antioxidant effects of flavonoids makes these molecules all the more interesting. Their ability to modulate neuroplasticity and neurogenesis means that not only could they treat the symptoms of dementia but, perhaps, they could actually reverse the damage caused by the disease. In addition, these neural effects could explain the beneficial effects of flavonoid intake in a number of other disorders beyond dementia. For example, flavonoids appear to improve mood in depression, a disorder associated with decreased levels of BDNF and reduced neurogenesis.

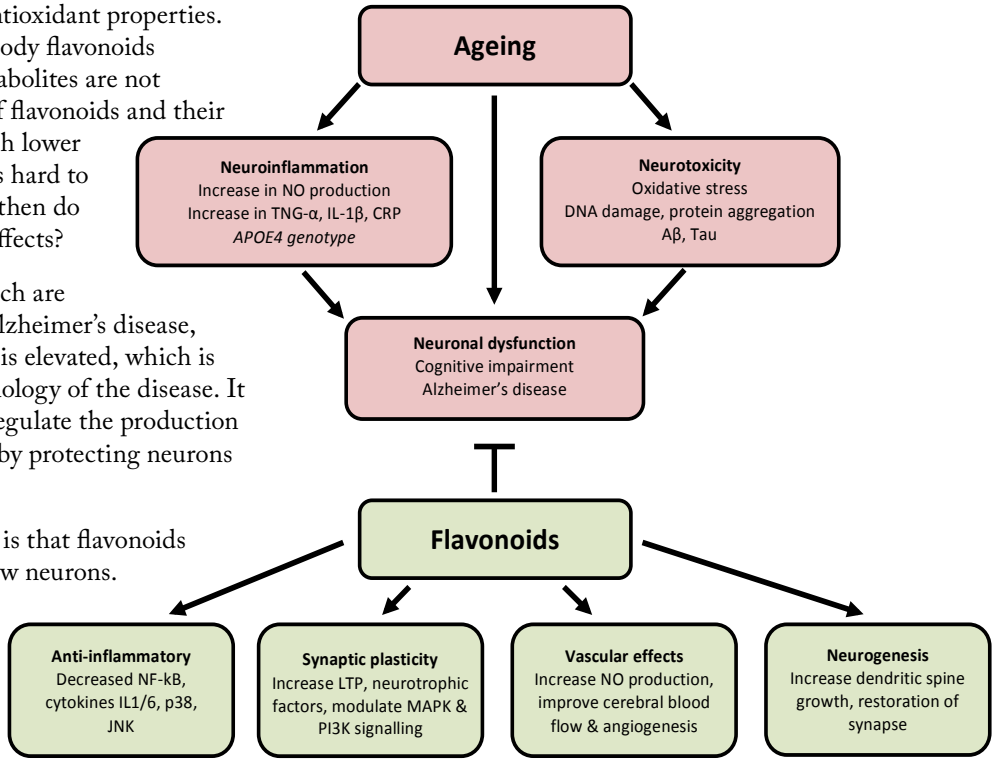


Figure 2: Potential neuroprotective mechanisms of flavonoids. Figure reproduced from (5), used with permission.

More than anything, research into the mechanisms of flavonoids *in vivo* demonstrates just how important diet can be in health. Amid the development of state-of-the-art therapies and exciting breakthroughs in drug discovery, it is easy forget how much we can learn about the treatment and prevention of disease by just looking at the food and drink that we consume every day.

References

1. Geleijnse JM, *et al.* (2002) Inverse association of tea and flavonoid intakes with incident myocardial infarction: the Rotterdam Study. *Am J Clin Nutr* 75(5):880-886.

2. Onozuka H, *et al.* (2008) Nobiletin, a citrus flavonoid, improves memory impairment and Aß pathology in a transgenic mouse model of Alzheimer's disease. *J Pharmacol Exp Ther* 326(3):739-744.

3. Commenges D, *et al.* (2000) Intake of flavonoids and risk of dementia. *Eur J Epidemiol* 16(4):357-363.

4. Okuyama S, *et al.* (2012) Heptamethoxyflavone, a citrus flavonoid, enhances brain-derived neurotrophic factor production and neurogenesis in the hippocampus following cerebral global ischemia in mice. *Neurosci Lett* 528(2):190-195.

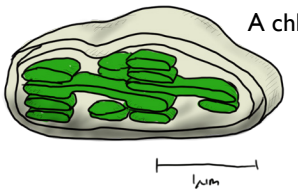
5. Vauzour D (2014) Effect of flavonoids on learning, memory and neurocognitive performance: relevance and potential implications for Alzheimer's disease pathophysiology. *J Sci Food Agric* 94(6):1042-1056.

Matthew Warren is a second year DPhil student in the Department of Psychiatry.

Tree *of* Plants

What do you imagine when you think of a plant? Leaves, flowers, seeds and roots? These are all actually quite recent developments in plant evolution. Without plants with seeds and fruits the world would be wildly different; essentially all crop plants have flowers and all have roots and leaves. So where did they evolve from?

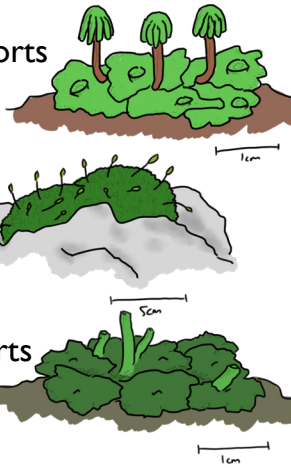
The story of plants starts about 1,600 million years ago (mya) with a cyanobacterium and a hungry unicellular eukaryote.



The **photosynthetic** cyanobacterium was eaten but survived to become an endosymbiont; the first chloroplast. Every single chloroplast in every photosynthetic eukaryote organism is derived from this one event.

Plant life began in the ocean with unicellular alga-like cells. **Multicellularity** evolved as many as three times, once making red algae and twice making two lineages of green algae. These multicellular algae are all very simple, with very little specialisation of cell function.

Now things get exciting! One type of green algae (the charophytes) got bored in the water and moved to the land. This happened around the same time that the algae evolved the ability to have cells with specialised functions; they were able to develop **tissues**.



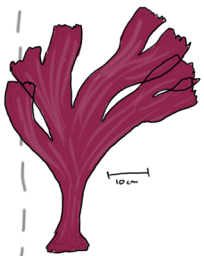
Charophytes
(more green algae)

Chlorophytes
(green algae)

Ulva lactuca
(sea lettuce)

Photosynthesis has been 'stolen' from red and green algae several times; diatom chloroplasts were derived from red algae endosymbionts.

Rhodophytes
(red algae)



Botryoglossum farlowianum

Glaucophytes
(unicellular green algae)

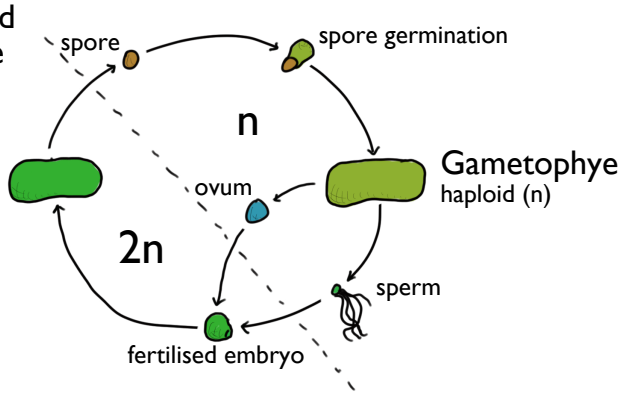
1,600 mya 1,400 mya 1,200 mya 500 mya

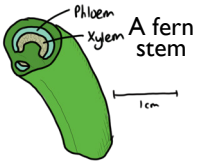
The 6 steps domination:

1 Photosynthesis
2 Multicellularity
3 Tissues

The first plants on land were strange compared to most modern land plants as, like bryophytes and algae, they spent most of their time as a haploid gametophyte. Ferns were a revolution, existing mainly as a diploid sporophyte.

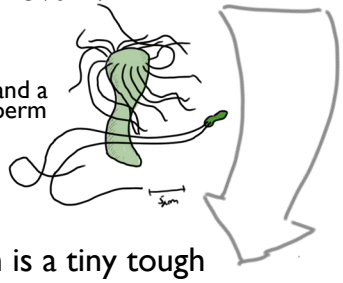
The generalised plant life cycle



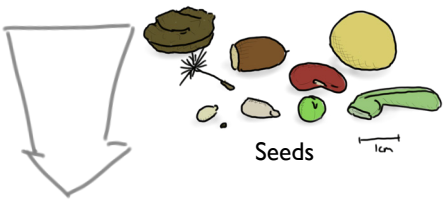


Fern sporophytes developed a **vasculature** system – biological tubes for water transport. This helped them survive in drier, harsher environments.

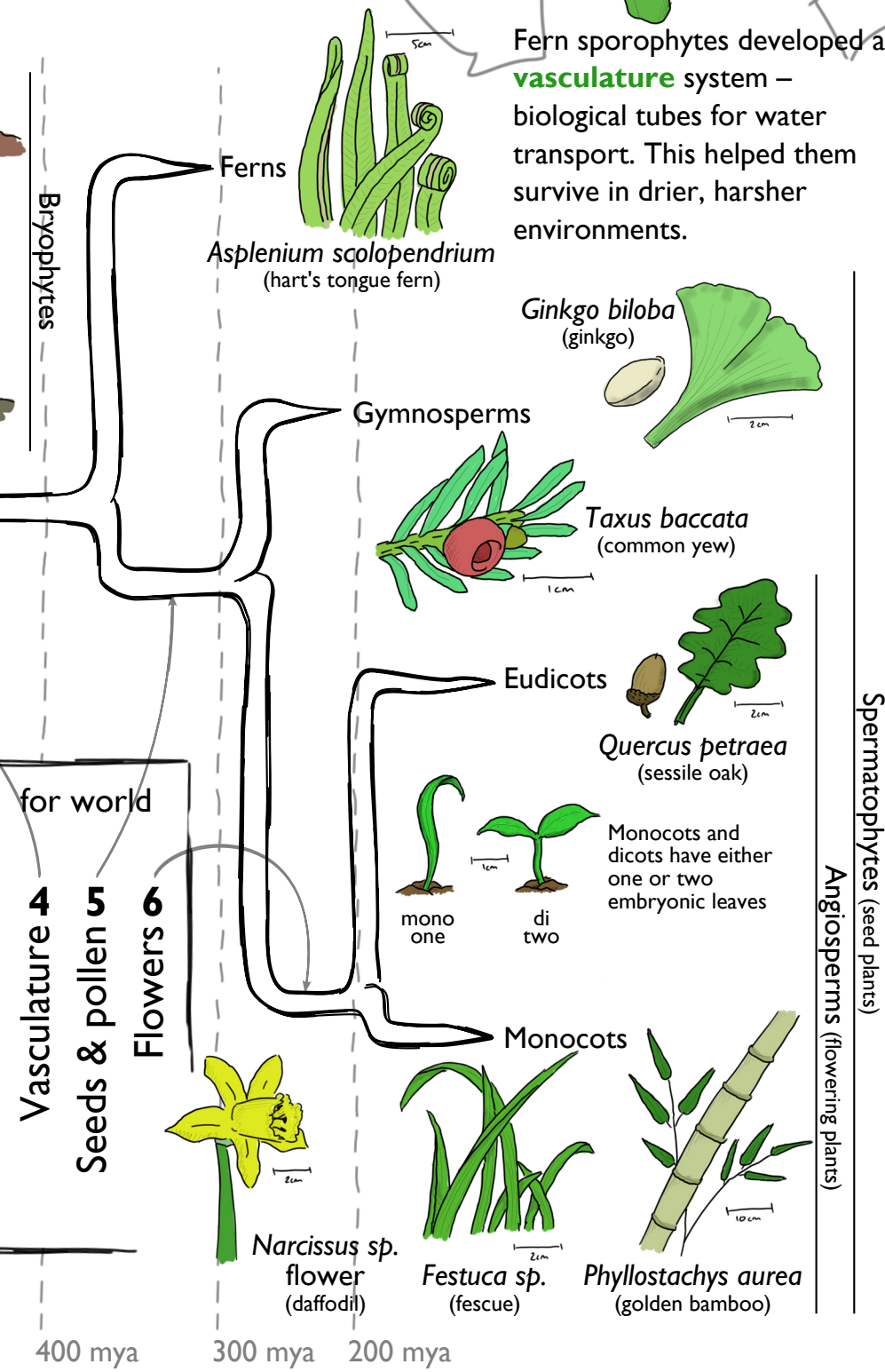
The final innovation that let plants truly conquer the land was **pollen**. Ferns and bryophytes still relied on water in the environment for their sperm to be able to swim and find an ovum.



Pollen is a tiny tough gametophyte which only produces sperm when it is right next to an ovum. Coupled with **seeds** (embryonic plants resistant to drying out) and **flowers** (to assist pollination) plants have minimised their dependency on water.



Seeing as essentially all food from plants comes from flowering plants (angiosperms) it is easy to forget about this diversity, and the history through which plants came to cover the Earth. It has taken millions of years to get from chloroplasts to rice!



By Richard Wheeler 2014

Bowman JL, *et al.* (2007) Green Genes – Comparative Genomics of the Green Branch of Life. *Cell* 129(2):229-234.

Nipping Alzheimer's in the bud:
How branching into *Arabidopsis* research can help get us to the root of human disease

by
Dr Ruth Faram

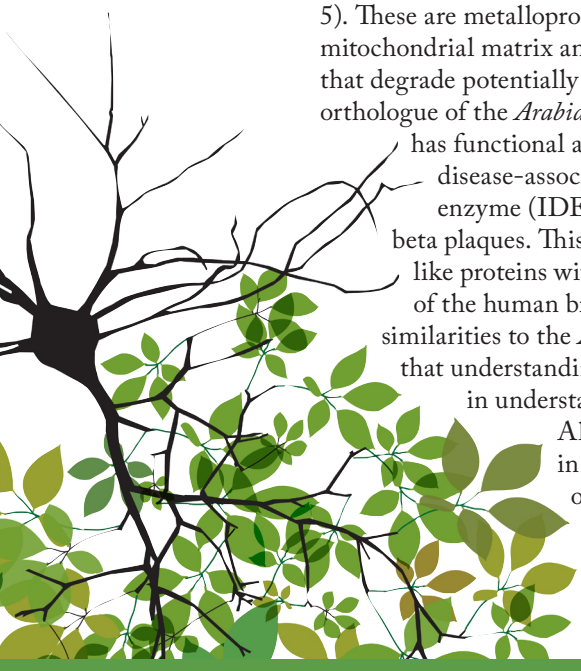
The plant species *Arabidopsis thaliana* is becoming increasingly used in research, not only by plant biologists but also by those investigating human disease. The Human Genome Project demonstrated conservation of a high percentage of genes between *Arabidopsis* and humans: 71% of genes implicated in human neurodegenerative disease have an *Arabidopsis* orthologue (1). This suggests that *Arabidopsis* may be of value as a model organism for studying human disease pathology.

Alzheimer's disease is the most prevalent neurodegenerative disorder, clinically defined by onset of senile dementia with gradual loss of cognitive and motor function. It is characterised by neuronal aggregation of amyloid beta protein plaques and tau protein neurofibrillary tangles, resulting in neuron toxicity and cell death. Three causal genes (*APP, PSEN1* and *PSEN2*) and one risk gene (*APOE*) have so far been identified (2). *APP* encodes the amyloid precursor protein, *PSEN1/2* encode presenilin and *APOE* encodes apolipoprotein E. BLAST analysis of genes associated with Alzheimer's has identified that most have orthologues within the *Arabidopsis* genome, some of which have defined functions (3).

Whilst physiological differences between *Arabidopsis* and humans may limit studies into disease pathology, this organism still provides an avenue for probing gene or protein function. This translational approach was demonstrated by the discovery of two proteases in *Arabidopsis*: PreP1 and PreP2 (4, 5). These are metalloproteases, localised within the mitochondrial matrix and chloroplast stroma (6), that degrade potentially toxic peptides. The human orthologue of the *Arabidopsis* PrePs is hPreP. hPreP has functional analogy to an Alzheimer's disease-associated insulin degrading enzyme (IDE), which degrades amyloid beta plaques. This suggests other PreP-like proteins within the mitochondria of the human brain may have functional similarities to the *Arabidopsis* proteases, and that understanding PreP activity may help in understanding disease pathology. Although hPreP is present in the Alzheimer's brain, it often fails to break down plaques. It is suggested that mitochondrial dysfunction, and consequent increased levels of reactive oxygen species (ROS) within the brain, may lead to inhibition of hPreP. Increased understanding of hPreP activity and alteration of mechanism in the Alzheimer's brain, including hPreP inhibition by ROS, may be gained by probing the originally discovered *Arabidopsis* PrePs.

Therefore, PrePs have an important role in mitochondrial function. Should they be disrupted, disease phenotypes can arise. Although hPreP is only one example of multiple proteins associated with disease states, these studies define the importance of molecular research using *Arabidopsis*. No matter what the species under investigation, the mechanisms underpinning molecular function can potentially be determined and translated to species where orthologues exist. *Arabidopsis* should no longer be considered solely a model for plant biologists, but also for those researching mammalian health and disease.

Image by Óscar Cordero Llana.



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References

1. Rubin GM, *et al.* (2000) Comparative genomics of the eukaryotes. *Science* 287(5461):2204-2215.
2. Bettens K, *et al.* (2010) Current status on Alzheimer disease molecular genetics: from past, to present, to future. *Hum Mol Genet* 19(R1):R4-R11.
3. Xu MX & Møller SG (2011) The value of Arabidopsis research in understanding human disease states. *Curr Opin Biotechnol* 22(2):300-307.
4. Alikhani N, *et al.* (2009) Mitochondria and Alzheimer's disease: amyloid-beta peptide uptake and degradation by the presequence protease, hPreP. *J Bioenerg Biomembr* 41(5):447-451.
5. Glaser E & Alikhani N (2010) The organellar peptidasome, PreP: a journey from Arabidopsis to Alzheimer's disease. *Biochim Biophys Acta* 1797(6-7):1076-1080.
6. Moberg P, *et al.* (2003) Characterization of a novel zinc metalloprotease involved in degrading targeting peptides in mitochondria and chloroplasts. *Plant J* 36(5):616-628.

Pressed for time

Dedicated individuals have created and used comparative collections to understand the natural world for centuries. At the core of such collections are specimens. These can be either preserved objects, such as rocks, fruits or bones, or living objects in botanic gardens, zoos, culture collections or gene banks. Today, such physical collections are augmented by the vast digital collections contained in molecular databases.

by Dr Stephen Harris

Left: Snakehead Fritillary collected by Jacob Bobart the Younger, second Keeper of the Botanic Garden, in c. 1680. **Right:** Lousewort collected by Carolus Linnaeus in Lapland and presented to Johann Dillenius following his visit to Oxford in 1736.

When Henry Danvers gave the University £5,000 in 1621 to establish a Botanic Garden, he perhaps saw its function as bringing together the "vegetable products of Creation" and increasing our understanding of them. The first Keeper of the Botanic Garden, Jacob Bobart the Elder, recognised that botanical research needed both living and dead plants from across the planet. Consequently, a collection of flattened, dried plants – a herbarium – was essential. Herbaria provide information about the identity and occurrence of species. They are central to initiatives to catalogue plant life on Earth, to understand plant evolution, and in discussions about the future of plant diversity in the face of global change. Oxford has Britain's oldest herbarium. It has roots in the Botanic Garden, opposite Magdalen College, but the whole collection moved to the Department of Plant Sciences in the 1950s. Worldwide, there are approximately 2,600 herbaria containing over 300 million specimens; Oxford Herbaria contains approximately one million of these.

Specimens are a permanent scientific record and, as such, are fundamental to taxonomic and ecological research. They are also often the only means of identifying plants in poorly investigated parts of the world. Specimens are still being added to the Oxford Herbaria and, while techniques for drying and preparing plants have changed little since the mid-sixteenth century, the quality of data associated with specimens has improved dramatically. Specimens are put to a multitude of uses, most of which would have been inconceivable to their original collectors. Data from specimens allows us to, for example, identify pollen grains from peat cores, model species range changes and investigate consequences of climate change on areas of high biodiversity. Specimens are also sources of DNA for species difficult to obtain, as well as being mines of information for researchers in the humanities and social sciences.

Oxford University Herbaria is replete with botanical treasures. These include some of the first collections made in North America, South Africa and Australia, collections made by Charles Darwin and Carolus Linnaeus and representatives of many species that are now extinct. Illustrious botanists have contributed to, and studied, specimens in the Herbaria over centuries, leaving their mark on its evolution. However, stewardship of such treasures has not always been at its best. For example, one of the world's oldest plant collections, made by the monk Gregorio da Reggio in 1606, lay abandoned and forgotten in the Botanic Garden for centuries. It was not until the late-nineteenth century that it was unearthed from "a pile of material in the coke-house".

On July 25th 2021, the Botanic Garden, Herbaria and Department of Plant Sciences celebrate their quadricentenary. In the lead up to the celebrations, 400 plants are being profiled in 400 weeks in 400 words, on the Plants400 website (http://herbaria.plants.ox.ac.uk/bol/plants400). After four centuries of assisting research and teaching at Oxford, the Botanic Garden and Herbaria are still in their prime.

Modern plant presses drying during a recent expedition in Brazil.

Dr Stephen Harris is a group leader in Plant Sciences and the Druce Curator of the Oxford University Herbaria.

Flipping the switches:
An engineered future of enhanced nitrate transport?

by
Prof Simon Newstead

The growth in human population expected in the next few decades will place an increasing burden on the ability of countries to feed their populations. By 2030, global demand for food is expected to increase by 40%. As only 15% of Earth's landmass is suitable for crop production, almost all of which is now in use, simply expanding production is unlikely to satisfy demand. Innovative solutions to this problem are being pursued, including the generation of engineered crops with improved nutrient uptake and retention properties. In theory, such plants would yield more biomass for the same energy input.

One of the candidates being investigated for engineering is the nitrate transporter NRT1.1. Nitrogen is an essential rate-limiting nutrient for plant growth and development and is usually obtained through the uptake of nitrate ($NO3^-$) from the soil. Nitrate uptake is controlled through the *NRT1* and *NRT2* gene families that encode low (K_M mM) and high (K_M µM) affinity nitrate transporters respectively. Sedentary species, such as plants, have evolved both low and high affinity systems as a way to counteract fluctuating nutrient levels in the environment. Interestingly, the *NRT1* and *NRT2* gene families share no sequence similarity, suggesting that these uptake pathways are biochemically distinct.

However, in 2003 it was discovered that NRT1.1, a member of the low affinity NRT1 family, has dual affinity for nitrate (1). In conditions of high nitrate availability in the soil (>1 mM) NRT1.1 behaves as a low affinity, high capacity transporter (K_M ~4 mM) (2). However, when nitrate levels fall below 1 mM, NRT1.1 is phosphorylated by the kinase CIPK23 on residue Thr101, located on the intracellular side of the membrane. The transporter then switches into a high affinity, low capacity state (K_M 40 µM) (3). This regulatory mechanism allows plants to alternate rapidly between low and high affinity nitrate uptake. These observations suggest a complex interplay between substrate affinity and post-translational modification at the molecular level. However, except for its effect on membrane trafficking and cellular localisation, very little is known about the detailed mechanism(s) by which phosphorylation regulates transporter function *in vivo*.

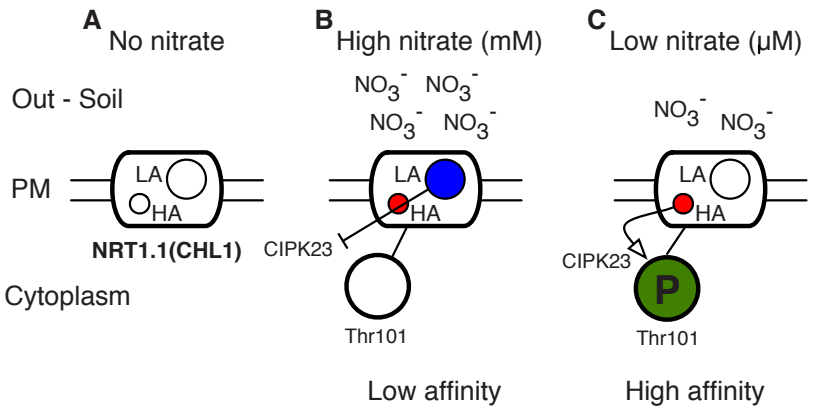

Figure 1: Schematic model for the regulation of nitrate binding affinity in NRT1.1. **(A)** NRT1.1 is thought to contain both high affinity (HA) and low affinity (LA) binding sites for nitrate, accessible to the outside of the cell. **(B)** Under high nitrate conditions both HA and LA sites are occupied, causing a block to CIPK23 mediated phosphorylation of Thr101 (open circle). **(C)** When nitrate concentrations drop, the LA site is no longer occupied, releasing the block on CIPK23, resulting in phosphorylation of Thr101 (green circle) and the switch to the high affinity state.

NRT1.1 is also reported to act as an extracellular nitrate sensor or receptor, controlling the primary nitrate response to changes in soil nitrate levels. When nitrate levels fall, NRT1.1 induces upregulation of the high affinity nitrate transporter NRT2.1. Interestingly, this function is distinct from that of transport, as a transport-deficient variant of NRT1.1, Pro492Leu, can still control the primary nitrate response in *Arabidopsis thaliana*. Membrane transporters that act as both nutrient transport and metabolic signalling receptors have been termed 'transceptors' in recognition of this dual role. Figure 1 shows a possible model for how NRT1.1 may function in nitrate transport and signalling. NRT1.1 is a key component of nitrate signalling and uptake in *A. thaliana,* and is likely to play an equally important role in commercial crops such as barley, wheat, rice and corn where close homologues exist (>90% sequence identity).

Crystal structure reveals nitrate-binding site

To understand more about the role of NRT1.1 in the nitrogen response, Dr Joanne Parker, a senior scientist in my group, successfully obtained the 3D crystal structure to 3.7 Å resolution in the absence and presence of nitrate (Figure 2) (4). NRT1.1 belongs to the Major Facilitator Superfamily of secondary active transporters and the structure revealed the canonical 12 transmembrane helices adopting the inward-facing state of the transporter in the membrane. In this state, the central ligand-

binding site is sealed on the extracellular side of the membrane but opens out towards the cytoplasm. In addition to the 12 transmembrane helices that constitute the transporter, NRT1.1 contains a large cytoplasmic domain consisting of 84 amino acids. Although the bulk of this domain was disordered in the crystal structure, a long helix was observed jutting out from the transporter domain, which we termed the 'lateral helix'. This domain and lateral helix may form part of the kinase-binding site. The identification of nitrate in the binding site allowed a detailed study of the binding site to be developed and the molecular basis for the dual affinity nature of this transporter to be determined.

Phosphorylation results in an increase in transport rate, not binding affinity for nitrate

A key question we wished to address was the molecular basis for the affinity switch reported previously for NRT1.1. In these experiments the authors had demonstrated that under low nitrate levels NRT1.1 is phosphorylated on Thr101. Furthermore, they showed that a Thr101Asp mutation faithfully replicated the effect of phosphorylation. Our structure provided evidence that Thr101 was situated near the intracellular face of the transporter and away from the nitrate-binding site (Figure 2). To test the effect of changing Thr101 to an aspartic acid, we developed an *in vitro* binding assay that employed microscale thermophoresis (MST). MST uses fluorescence to measure the change in thermal diffusion of a biomolecule as a function of ligand concentration. In our assay, we labeled NRT1.1 with a C-terminal GFP protein and measured the effect of nitrate concentration on diffusion. We discovered that the Thr101Asp mutant had no effect on the K_D for nitrate binding. We further validated the assay by showing that a His356Ala mutation abolished nitrate binding, consistent with our crystal structure data.

So how was phosphorylation changing the K_M for nitrate? To investigate this question further we developed an *in vitro* transport assay using liposomes. Our results showed that in the Thr101Asp variant, nitrate uptake was approximately four-fold higher than the wild-type protein. Investigations into the thermal stability of NRT1.1 using circular dichroism further revealed that the Thr101Asp protein was also considerably less stable than the wild-type by approximately 9°C. Our structure reveals that the Thr101 side chain sits in a small hydrophobic pocket within the N-terminal transmembrane helices, where the addition of a large phosphate group would cause substantial disruption to the helix packing. Our current hypothesis is that this disruption causes NRT1.1 to cycle faster in moving nitrate across the membrane but at the expense of structural stability.

Future perspectives

Our study has led us to propose the molecular basis for nitrate uptake by NRT1.1. However, NRT1.1 also acts as nitrate receptor, switching on the dedicated high affinity nitrate transporter *NRT2* family following phosphorylation at Thr101. A key aim of our future research is to understand how NRT1.1 is able to regulate the expression of *NRT2* genes.

Once we have understood this mechanism, can we then begin to design innovative solutions to manipulate nitrate uptake in commercial crops? We are confident our work on NRT1.1 shows that molecular insight into these fundamental processes is possible and that biochemistry will play a key role in addressing this increasingly important question as the demand for food production rises.

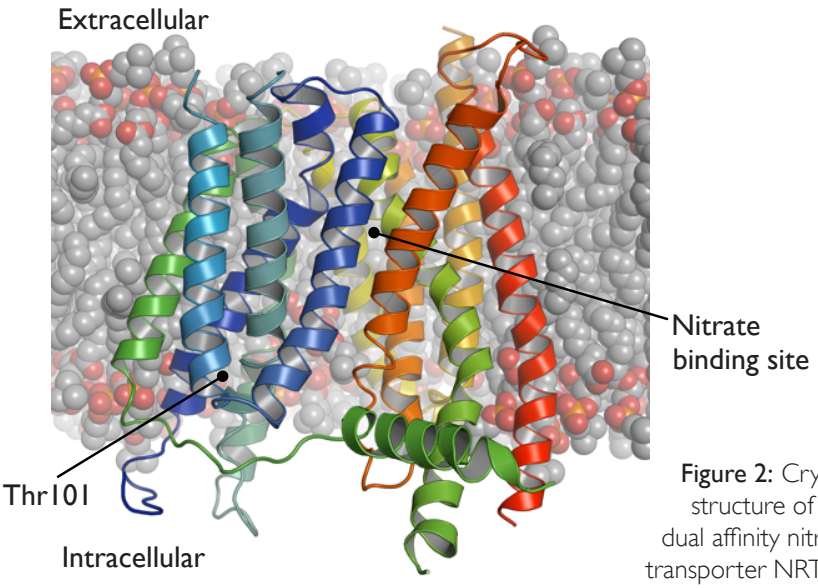

Figure 2: Crystal structure of the dual affinity nitrate transporter NRT1.1 from *Arabidopsis thaliana* (RCSB PDB entry 4CL5), represented in a POPC lipid bilayer, courtesy of Dr Phillip Stansfeld.

References

1. Liu K-H & Tsay Y-F (2003) Switching between the two action modes of the dual-affinity nitrate transporter CHL1 by phosphorylation. *EMBO J* 22(5):1005-1013.

2. Liu K-H, *et al.* (1999) CHL1 is a dual-affinity nitrate transporter of *Arabidopsis* involved in multiple phases of nitrate uptake. *Plant Cell* 11(5):865-874.

3. Ho C-H, *et al.* (2009) CHL1 functions as a nitrate sensor in plants. *Cell* 138(6):1184-1194.

4. Parker J L & Newstead S (2014) Molecular basis of nitrate uptake by the plant nitrate transporter NRT1.1. *Nature* 507(7490):68-72.

Prof Simon Newstead is an Associate Professor and group leader in Biochemistry.

Rice in a flood:
Could traditional selective breeding help us out of deep water?

by Sofia Hauck

Cultivation of rice began at least 10,000 years ago. Today, a dizzying array of genetic diversity is present in the domesticated *Oryza sativa* species and its dozens of wild relatives. There are over 150,000 rice cultivars – varieties developed by selective breeding – many of which are adapted to manage various types of flooding, a condition prevalent in many rice-producing areas. By what mechanism does this work and what impact does this knowledge have for the impending World Food Crisis?

Rice is a semiaquatic plant and can cope well with partial submergence. However, like all flowering plants, it is aerobic and cannot survive total submergence. Luckily for the millions that depend on rice as their staple crop, many cultivars are adapted to manage various types of flooding. In fact, the main classification of cultivars is based on their response to submergence (Figure 1). Upland rice does not have a strong response, because it is grown in areas not prone to flooding. Lowland rice is occasionally subject to flash floods, which may submerge the entire plant for several days. Deep-water rice, meanwhile, is adapted to floods that may last for months.

Lowland and deep-water rice varieties take different approaches to flood management. Lowland rice has 'submergence tolerance': when it detects a flood, it braces itself to wait out the inclement weather. It can survive relatively unscathed through 10 days of complete submergence by reducing its oxygen demand and using starch reserves for energy. Deep-water rice, like its name suggests, is required to persevere through much longer than a week's flooding: up to five months of over a metre of water. Instead of tolerance, it survives through 'submergence avoidance': when flooding is detected, the plant makes an all-or-nothing push for the surface, rapidly elongating in the hope of avoiding total submergence (1). Its tactic earns it the alternative name of 'floating rice'.

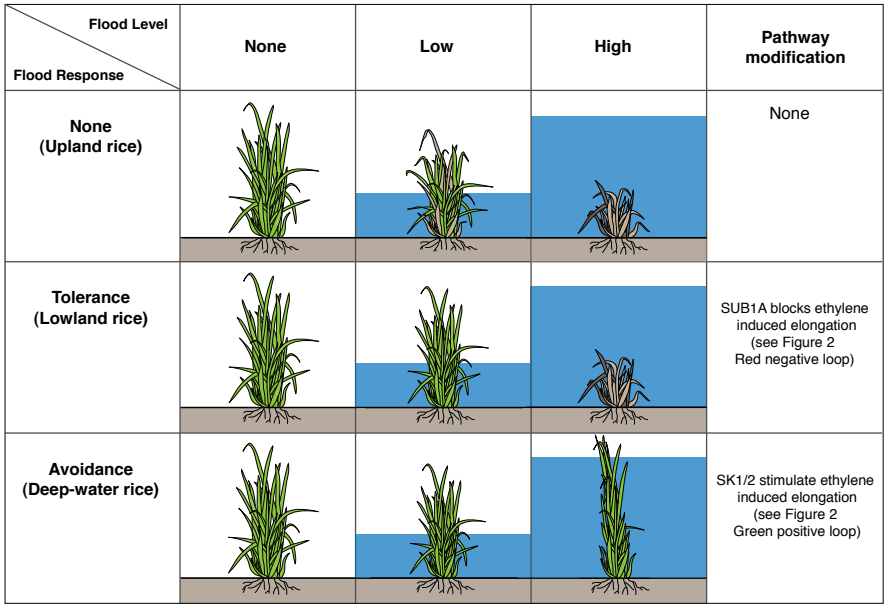

Figure 1: A pictorial summary of how the three types of rice cultivar fare during different levels of flooding. Modifications to the flood response pathway (Figure 2) leading to submergence tolerance or avoidance are also summarised. Images by Sharon Ruane.

Neither of these strategies comes without cost. Tolerance requires sufficient starch reserves to be present before flooding occurs, in order to maintain basic functions whilst submergence persists. Without these reserves, the plant will die. Avoidance, meanwhile, carries a severe risk. If the flood is too deep, the rapid elongation response, which can reach up to 25 cm per day, may mean the plant engages in suicidal growth, depleting all of its reserves in a few days. Even if the elongation response is successful in escaping water, a sudden drop in water level sometimes causes the plant to keel over, unable to support its own weight due to the weak foundations laid during its desperate growth spurt.

Thanks to the tireless work of plant scientists since deep-water rice was first brought to the lab in the 1950's, we now understand the genetic mechanisms of submergence tolerance and avoidance. When a rice plant is submerged, ethylene builds up in tissues due to slower diffusion to the environment (Figure 2). Ethylene inhibits abscisic acid, which itself inhibits gibberellic acid, meaning that an increase in ethylene leads to a larger gibberellic acid response. This includes growth elongation. Submergence tolerance depends on a modification of the *SUB1* locus, which controls up to 70% of phenotypic variation in this trait. *SUB1A*,

present in submergence tolerance cultivars, encodes ethylene responsive factor DNA binding proteins, which block ethylene from activating the pathway that leads to growth elongation (Figures 1 and 2).
Deep-water rice's avoidance strategy is controlled largely by the *SNORKEL* loci, *SK1* and *SK2*. These two loci appear to be entirely absent in non-deep-water varieties, and, like *SUB1*, code for ethylene responsive factor DNA binding proteins. However, they have the opposite effect. They trap ethylene and increase its concentration, resulting in degradation of abscisic acid and increased gibberellic acid levels (Figures 1 and 2). Gibberellic acid promotes cell elongation and division in the stem, and activates *SUB1C,* which drives use of the plant's starch reserves for growth. The two submergence responses, tolerance and avoidance, therefore exist as changes to the same point in the same pathway: the concentration of ethylene. Consequently, they cannot both be present in one cultivar and represent a choice that must be made during cultivar development.

As early as 1993, *SUB1A* was bred into high-yield semi-dwarf rice cultivars. *SUB1A* and a mutated *SUB1C* were later introduced into several modern high-yield varieties. Since 2010, these 'Sub1 mega-varieties' have been released for agricultural use in India, the Philippines, Indonesia and Bangladesh. As *SUB1* only modifies growth during flooding, these changes have no adverse effects on development, yield or grain quality under normal conditions. If flooding does occur, Sub1 mega-varieties can produce three to six times more grain by weight than their non-Sub1 parents. Previously, all commercially important cultivars were intolerant to complete submergence, which highlights the positive impact of Sub1 mega-varieties in areas prone to flooding.

The success of these flood resistant crops is all the more promising considering the failure on a grand scale of the Golden Rice Project. In 1999, Prof Ingo Potrykus, together with his Swiss and German colleagues, developed a proof-of-concept rice cultivar capable of producing provitamin A. Vitamin A deficiency causes thousands of children to die or be permanently blinded each year. Golden rice promised to ameliorate this issue, and giving seeds to those in need promised to help them solve their own problems. As a convergence of cutting-edge science and humanitarian aid, the Golden Rice Project attained recognition in mainstream media, including a cover story in a July 2000 edition of *Time* magazine. However, it is now 2014 and no golden rice is being planted for consumption anywhere in the world, although there are some ongoing field trials (3, 4). The insurmountable barrier was not intellectual property rights or lack of funding, but legal

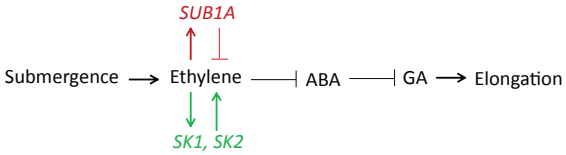

Figure 2: The pathway activated by a build-up of ethylene during submergence. ABA = abscisic acid and GA = gibberellic acid. Modifications to the pathway allow submergence tolerance (red negative loop) or avoidance (green positive loop).

regulation of genetically engineered crops. Such laws vary regionally and often have multiple layers of complexity. At their crux, they suggest that field trials are unsafe because a genetically modified cultivar is untested. However, it is only possible to test a cultivar in a field trial. Exceptions to this catch-22 come only in limited and often expensive circumstances, such as conducting massive trials in complete containment. These issues have delayed deployment of golden rice by at least a decade (5).

The difference between flood resistance and provitamin A production is that the former is a trait present amongst rice cultivars. None of the 80,000 cultivars catalogued so far can produce provitamin A – a very visible trait that dyes the grains a yellow colour, giving golden rice its memorable moniker. Sub1 mega-varieties were created using modern techniques to screen crosses, but never to directly add to plants' genomes, and therefore are not considered genetically engineered. Traditional breeding methods like these are limited by the existing diversity of the species in question. In rice, that diversity is huge, but it is not infinite. We must choose whether our concerns over safety and the 'unnatural' overrule the benefits of rapidly and dramatically improving crops. If that is our choice, we cannot forget that traditional breeding techniques still have much to offer.

References

1. Sauter M (2000) Rice in deep water: "How to take heed against a sea of troubles". *Naturwissenschaften* 87(7):289-303.

2. Bailey-Serres J, *et al.* (2010) Submergence Tolerant Rice: *SUB1*'s Journey from Landrace to Modern Cultivar. *Rice* 3(2-3):138-147.

3. Enserink M (2008) Tough lessons from golden rice. *Science* 320(5875):468-471.

4. Mayer J (2014) Golden Rice Project. Available at http://www.goldenrice.org [Accessed March 2014].

5. Potrykus I (2012) "Golden Rice," a GMO-product for public good, and the consequences of GE-regulation. *J Plant Biochem Biotechnol* 21(S1):S68-S75.

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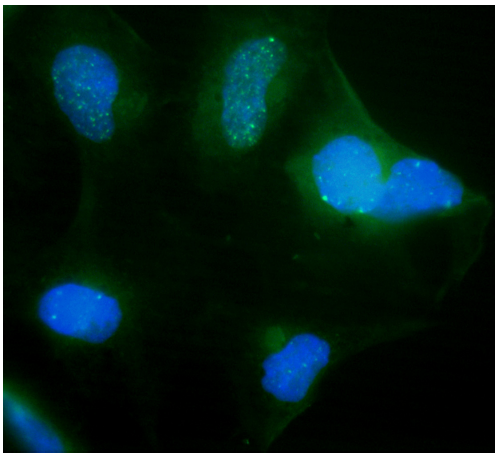
From the forest to the clinic:
Natural plant compounds as anti-cancer treatment

by
Dr Ross Cloney and Kelvin Chan

The natural world and the bounty of the forest have long been sources of medicines for the cultures that recognised and harvested their potential. One of the best known examples is that of the willow tree. Its key active compound, salicylic acid, was first mentioned in Ancient Greek and Egyptian texts as a treatment for fever. A derivative of salicylic acid is now mass manufactured as aspirin. One of the areas where interest in plant-derived compounds is particularly strong is the search for new chemotherapeutic drugs in the treatment of cancer.

As our understanding of cancer has improved, we have come to recognise that cancer is not so much a single disease as a varied set of diseases that share a common set of characteristics. Cancer is a 'disease of the genes': the accumulation of genetic errors, hastened by biological predisposition or lifestyle choices, that results in a population of cells freeing itself from the growth restraints imposed on healthy cells. Cancers display a fantastic range of genomic heterogeneity between people, tissue type and even among cells in the same tumour. However, all cancers are defined by ten hallmarks: dysregulated metabolism, replicative immortality, insensitivity to anti-growth signals, self-sufficiency in growth signals, the ability to evade apoptosis, genomic instability, the ability to evade immune system detection, sustaining an inflammatory environment, promoting angiogenesis and, particularly in highly aggressive cancers, metastasis (1).

Since cancer is caused by the accumulation of mutations, it is primarily a disease of the elderly for the simple reason that they have had longer to build up a critical level of genetic errors. Currently, one in three people are predicted to be diagnosed with cancer in their lifetime with the expectation that as the population ages, the rate will rise to one in two (2). With increasing knowledge of lifestyle choices that reduce the risk of cancer and the identification of risk factors in the population, such as testing for a *BRCA1* or *BRCA2* mutant allele in women with a family history of breast cancer, success rates for survival are increasing year by year (3). However, different cancers have different levels of success for treatment outcome. Several cancers are still highly difficult to treat and illustrate the importance of developing novel chemotherapy drugs.



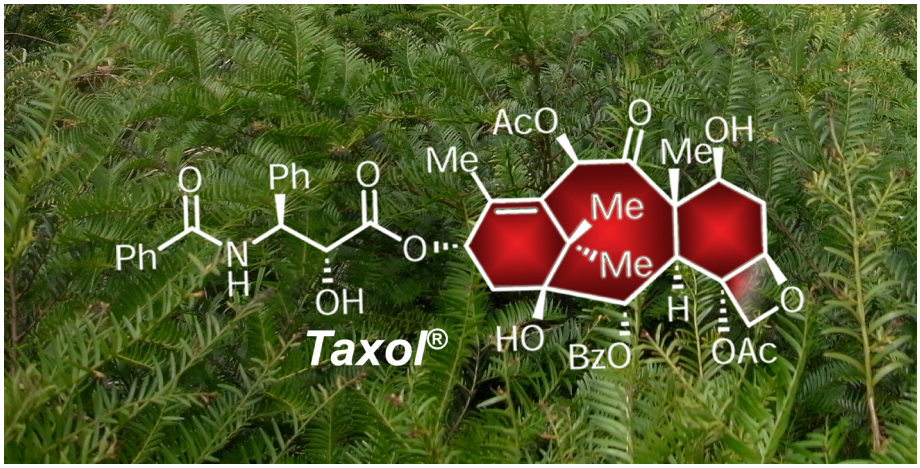
Human cancer cells displaying markers of DNA damage. Image by Ross Cloney.

Several natural plant compounds have already been isolated from their sources and put to use in the laboratory and the clinic. Paclitaxel (Taxol®) is a famous natural plant-derived compound, widely used in the UK for treatment of lung, breast, ovarian and other solid state tumours. Originally derived from the bark of the Pacific Yew Tree *Taxus brevifolia*, it is now understood that a symbiotic fungus in the bark produces the drug. Taxol functions as a microtubule stabiliser, preventing the depolymerisation of the established cytoskeleton and the formation of the spindle structures required for mitosis. These are both required in proliferating cells, including rapidly dividing cancer cells. With their microtubules locked into an artificially stable structure, cells cannot appropriately segregate their genetic material and finish mitosis, leading to cell death. Interestingly, Taxol functions in the opposite manner to a wide range of chemotherapy drugs that prevent cell division by destabilising microtubule structures.

The story of Taxol is illustrative of the path taken by natural compounds as they progress from discovery to the clinic and the care that must be taken in harvesting naturally occurring compounds. The journey begins in 1962 with samples from *Taxus brevifolia* being harvested by Arthur S Barclay as part of the American National Cancer Institute initiative to identify novel plant compounds. Of the 110,000 compounds identified by the survey, Taxol showed the most promise but was ignored until 1979 when Susan Horwitz's team demonstrated its microtubule-stabilising properties in a key Nature publication (4). The anti-cancer potential combined with the environmental impact of harvesting led to a flurry of interest from chemists keen to synthesise the drug. The problem was cracked by the Nicolaou group in 1994, and was followed by a succession of alternative synthesis pathways throughout the mid-90s (5).

One of the key discoveries for securing a long-term sustainable supply of Taxol was the identification

of closely related plant compounds. The Yew tree was unsuitable for the large-scale harvesting that the widespread use of a promising anti-cancer drug would require. The tree grows slowly in difficult-to-access forest ecosystems, and large-scale manufacturing of Taxol to treat 12,000 patients for clinical trials required the sacrifice of 38,000 trees. The discovery that a closely related tree, *Taxus baccata* or the common European Yew, could be used to isolate the closely related starting compound 10-deacetylbaccatin III from its needles without killing the tree allowed the widespread adoption of this potent anticancer drug. Today, Taxol and related compounds are mass-manufactured industrially and are leading compounds in the anti-cancer market.



The molecular structure of Taxol® with a Chinese Yew Tree. Image by KC Nicolaou, used with permission.

As our finesse with chemotherapy matures, is there still a role for natural plant compounds in the clinic or will they be supplemented by targeted, designed drugs based on our knowledge of the molecular biology of the cell? We were fortunate enough to be able to directly ask a leading researcher in the field, Prof Phil Baran of The Scripps Research Institute, for his insights into the future of natural product synthesis and cancer therapeutics.

RC & KC: You currently consult for numerous pharmaceutical companies; how much interest does pharma still have in identifying natural products as a starting point for general drug discovery?

PB: They are making a comeback. I think the trend these days is to focus on pursuing validated biological targets that will lead to positive clinical outcomes. Once a company has identified that, most are pretty agnostic as to the type of small molecule that will get the job done. Advances in the synthesis of complex natural products are making medicinal chemists more open to using them as a starting point for drug discovery. The surge of interest in antibody-drug conjugates is also fuelling a renewed focus on natural products in some companies.

RC & KC: Much of your chemistry illustrates practical aspects of natural product synthesis, including scalable reactions. How successful do you think the synthetic community has been in synthesizing viable quantities of natural plant products that can be used towards discovery of novel chemotherapy drugs?

PB: The community has a long way to go. The era of feasibility demonstrated that anything is possible but now we have entered the age of practicality where the value of a synthesis is measured in terms of one's ability to make large quantities in an economically viable fashion (in terms of time, effort and cost of goods). Slowly but surely, the community will demonstrate that it is possible to routinely access viable quantities of natural products (derived from marine or terrestrial sources).

RC & KC: There is significant attention in the scientific literature concerned with analogues and structure-activity relationships. What are your thoughts about the direction of the field of new drug discovery – towards analogues or towards new natural products?

PB: I don't think it's one or the other. It's never wise to dismiss one area of science as being better or more likely to succeed than another. In my view, one needs to apply common sense in the interrogation of a biological target and be agnostic as to the small molecule starting point.

References

1. Hanahan D & Weinberg RA (2000) The hallmarks of cancer. *Cell* 100(1):57-70.

2. CRUK (2012) Lifetime Risk of Cancer. Available at http://www.cancerresearchuk.org/cancer-info/cancerstats/incidence/risk/ [Accessed March 2014].

3. CRUK (2013) Cancer Mortality for Common Cancers. Available at http://www.cancerresearchuk.org/cancer-info/cancerstats/mortality/cancerdeaths/ [Accessed March 2014].

4. Schiff PB, *et al.* (1979) Promotion of Microtubule Assembly *in vitro* by Taxol. *Nature* 277(5698):665-667.

5. Nicolaou KC, *et al.* (1994) Total synthesis of taxol. *Nature* 367(6464):630-634.

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Internalising radiotherapeutics that target oncogenic stress

by Christopher Hillyar

Cancer deaths are mainly caused by metastases, which are diverse in the targetable surface antigens they present and are difficult to treat with external beam radiation compared to primary tumours. Radiotherapeutics are anti-cancer molecular agents that consist of a radionuclide tagged with a targeting vector engineered to target both primary and metastatic tumour cells. When radionuclides decay, they emit energetic particles that break chemical bonds in biological material. The targeting vector, which may be an antibody, peptide, oligonucleotide, small molecule or nanoparticle, is linked to the radionuclide via an aromatic group or indirectly via a chelator. The vector recognises molecules specifically upregulated in cancer cells. For example, oncogenic stress results in DNA damage and thus the recruitment of DNA damage repair machinery. Radiopharmaceuticals can be targeted to these sites where the resulting ionisations inside the nucleus cause irreparable DNA damage, triggering apoptosis.

The specific properties of radionuclides vary according to their energy and mass, which ultimately affect the concentration of ionisations released along their path, a term known as linear energy transfer (LET). Let us consider some of the radiotherapeutic options. The radiohalogen iodine-131 (^{131}I) and the radiometal yttrium-90 (^{90}Y) both emit radiation in the form of low-LET beta particles, which deposit ionisations in low concentrations over long path lengths in tissue (1.3–11.0 mm) due to their high energy and negligible mass. Astatine-211 (^{211}At) and bismuth-213 (^{213}Bi) are both radiometals that emit high-LET alpha particles (helium nuclei), which, despite their high energy, deposit ionisations in high concentrations over short path lengths in the order of just a few cells (60–84 μm) due to their relatively large mass. However, the radiometal Indium-111 (^{111}In), emits high numbers of high-LET-like Auger electrons (up to 15 per decay), which collectively deposit ionisations in high concentrations due to their very low energy. Their very short path length ranges from the width of the DNA helix (2 nm) to the diameter of the nucleolus (several μm), making Auger electrons ideal for targeting DNA.

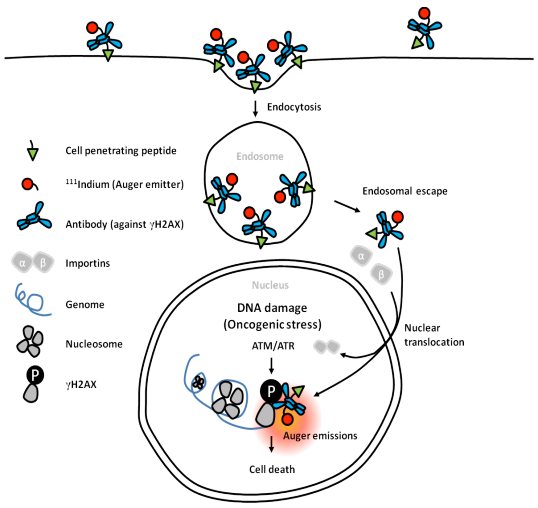


Figure 1: Targeting γH2AX with radiotherapeutics. Cell penetrating peptides promote endocytosis, endosomal escape and nuclear translocation of Auger-emitting radiotherapeutics which trigger apoptosis after binding γH2AX at DNA damage sites upregulated in cancer. Figure by Christopher Hillyar.

Auger electron-emitting radiopharmaceuticals must be internalised into cancer cells to reach their target sites (Figure 1). Endocytosis is triggered by either the binding of the targeting vector to its cognate membrane-associated receptor or, if a cell penetrating peptide (CPP) is used, embedding in the cell membrane. Clathrin-coated pits form at the site of membrane association, triggering the formation of endocytic vesicles. Aided by the positively-charged amino acid motifs of the CPP, radiopharmaceuticals escape from the endosome into the cytoplasm, avoiding degradation by the endolysosome. In the cytoplasm, radiopharmaceuticals containing a nuclear localisation sequence within their CPP or bound receptor are recognised by the alpha- and beta-importin proteins. The importin-radiopharmaceutical complex is translocated into the nucleus where the radiopharmaceutical can bind components of the DNA damage response (DDR).

A successful example employed ^{111}In-labelled anti-γH2AX antibodies, which bind to the histone variant H2AX only after it is phosphorylated by the DDR kinases ATM and ATR. ^{111}In decay emits Auger electrons that deposit ionisations at γH2AX sites causing complex DNA damage that overwhelms the DDR and therefore blocks tumour development (1). The very short range of Auger electrons means they are unlikely to reach the nucleus of non-malignant cells, although they may produce effects at the cell membrane (2).

^{111}In -labelled anti-γH2AX antibodies are one of several promising radiotherapeutic agents that seek out and eliminate metastatic cells undergoing oncogenic stress. Their development brings a cure for cancer ever closer.

References

1. Cornelissen B, *et al.* (2013) Molecular radiotherapy using cleavable radioimmunoconjugates that target EGFR and γH2AX. *Mol Cancer Ther* 12(11):2472-2482.
2. Pouget JP, *et al.* (2008) Cell membrane is a more sensitive target than cytoplasm to dense ionization produced by Auger electrons. *Radiat Res* 170(2):192-200.

Christopher Hillyar is a 2nd year DPhil student in the CRUK/MRC Oxford Institute for Radiation Oncology within the Department of Oncology.

by Lydia Le Page

Sense About Science

In 2012, activists from *Take the Flour Back* threatened to destroy genetic modification (GM) research at the Rothamsted Research Centre, UK. This is the longest-running agricultural research institute in the world and has been working on crop productivity and sustainable food production solutions for 170 years. Recently, coverage of GM crops has brought plant science to the front pages of the media. Public fervour surrounding genetic modification, fuelled in part by sensationalist reporting, has created restless activists who are threatening the advancement of science.

Sense About Science, a small London-based charity, is dedicated to furthering public understanding of science. Their remit – "equipping people to make sense of science and evidence on issues that matter to society" – underlies the multitude of activities they carry out. By talking to scientists and journalists, they facilitate sensible and accurate communication of current research. Working with scientists around the country, including some here in Oxford, they produce public guides that aim to break down complex topics such as GM, drug side effects, radiation and climate for the lay reader. One of their campaigns, which falls under the *Ask for Evidence* banner, encourages the public to engage in science by demanding more than just the word of a politician, newspaper editor or organisation.

Sense About Science aims to inform the public on scientific research using accessible and interactive formats.

In response to the anti-GM activists at *Take the Flour Back*, *Sense About Science* worked with scientists from Rothamsted to make a public video appealing to the activists to engage in discussion rather than destruction. Over 6,000 members of the public signed a petition in support of the research, open letters to activists were published and the conversation was widely covered in the media. Come protest day, turnout was low and the protest peaceful. This suggests that once presented with the aims of the research, the methods used and a clear discussion of the risks, public trust can be regained. This example illustrates the success of *Sense About Science*'s public-led, expert-fed approach.

GM hit the headlines again in 2013 due to the retraction of the controversial paper by Séralini *et al.* (1), which claimed to show increased tumour development in rats fed with GM corn. The authors stood by their results but were widely criticised for the choice of animal model, small number of animals used in the study, and questionable statistical analyses. When the study was first published in 2012, *Sense About Science* had provided an independent scientist's assessment of the paper. In 2013, they realised that there were more questions to be answered and there was a clear need for better public understanding regarding animal feeding studies. In order to address this, they ran a live Q&A with plant scientists to respond to queries from the public sent via email and Twitter. Scientific communication is paramount to publicity and promotion of the work done in laboratories around the world. It is key to engage the public and policy makers alike so that our research makes a difference to society. In addition, easily accessible science helps to secure research funding from funding bodies and from the government via the UK budget. Public understanding of science is also required to recruit future scientists, inspire our children and support the ethical arguments for animal testing. Misunderstandings can be costly.

An example of the negative effect of misrepresented science is the field of homeopathy. In 2009, a group of young scientists noticed that homeopathic remedies were being advertised for treating common diseases in Africa, such as malaria, HIV and tuberculosis. Working together with *Sense About Science*, they wrote an open letter to the World Health Organisation (WHO) asking them to condemn this practice. After wide-reaching media coverage, WHO stated that they did not recommend homeopathy as a replacement for evidence-based medicines. The enthusiasm of these young researchers made a global difference. They were part of *Sense About Science's Voice of Young Science* network (VoYS) – a group of early career scientists standing up for their research. They are myth-busters, evidence hunters and campaigners.

If you are an early career researcher and feel strongly about the portrayal of science in the media or want to join the push for evidence-based claims and policies, you too can join VoYS in standing up for science. Sign up online (2) to hear about the latest campaigns and how to take part, volunteer your scientific expertise or attend one of the VoYS workshops that are held around the country.

Lydia Le Page is a DPhil student in Physiology, Anatomy and Genetics and interned at Sense About Science in 2013. @_llepage

References

1. Seralini GE, *et al.* (2012) RETRACTED: Long term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize. *Food Chem Toxicol* 50(11):4221-4231.

2. Sense About Science (2014). Available at http://www.senseaboutscience.org [accessed 28 March 2014].

A career as a patent attorney

by
Dom Icely

If you had asked me about Intellectual Property (IP) in the first year of my undergraduate degree, I probably would have thought you were talking about clever estate agents. An inspiring talk at an Oxford University Biochemical Society careers event soon put me straight. Fifteen years on and working in IP, the field now has a higher profile, not least because of the recent 'Smartphone wars' between Apple and Samsung.

The following details are from my personal experience of being a patent attorney over the past decade. The field is rapidly changing, so if you are considering moving into this area some extensive research on the web is advised!

How did I get in?
At the University of Oxford Careers Service, I had a go at one of the assessment programs in order to find a career path that suited me. I was interested in science, but did not want to stay in the lab: a PhD just didn't excite me. Amongst the outputs that the computer gave me, which included postman, dustman and banker, the program suggested patent attorney. I wrote targeted letters to pretty much every practice in the South of England. While more information is available online these days, I would still recommend visiting the Careers Service and contacting companies with enquiries.

> "All of these apply: challenging, interesting, reasonable hours, and good pay."

When should you apply?
Although practices looking to recruit a graduate will generally aim for a September start date, most practices recruit throughout the year, so get your CV on record whenever you can. If you are an undergraduate in your final year, do it sooner rather than later as there may already be interviews in June. Mine was three days after the end of term.

Do you need a PhD?
There does seem to be a trend towards recruiting postgraduates, but undergraduates are often taken on.

What skills do you need?
In your CV, emphasise modules that you have studied that are more commercially relevant, such as immunology, pharmacology or human disease. A good example area to highlight would be an understanding of the immune response, given the importance of biologic drugs, such as antibodies. In addition to a solid scientific foundation, communication skills are one of the most important criteria. The ability to understand the nuances in one set of words compared to another when both seem to describe the same thing is crucial.

Determination is key: the exams are numerous and not trivial. You may not have failed an exam before, but you are statistically unlikely to sail through the law exams without one or more slip-ups. The good thing is that you don't need any legal expertise to start training – you will learn it all on the job.

Being presentable and confident with clients, and able to deliver on tight deadlines are also required.

What is the job like?
All of these apply: challenging, interesting, reasonable hours, and good pay. Sometimes you need to really engross yourself in the paperwork. Other times, you will have to think on your feet and present a case at a hearing. On one hand, it is technical, on the other, commercial. Linking the two is your knowledge and application of the law.

The core of the job is drafting and prosecuting patent applications. This involves understanding a new technology, seeing its commercial value and then carefully crafting a document describing the essence of the invention and its broader applications. You then need to fashion strong arguments, but also know when to give ground. The other side of the job is assessing others' patents, such as for infringement, so the same skills are applied, just in reverse.

I have really enjoyed my career so far and if you want to stay in touch with commercial science and enjoy the odd wrangle, I cannot recommend it enough!

Dom Icely is an alumnus of Molecular and Cellular Biochemistry (1996–2000) and has been in working in patent attorney practices throughout his 13-odd years in the profession, first at Marks & Clerk and now at IP Asset.

Museum of Natural History

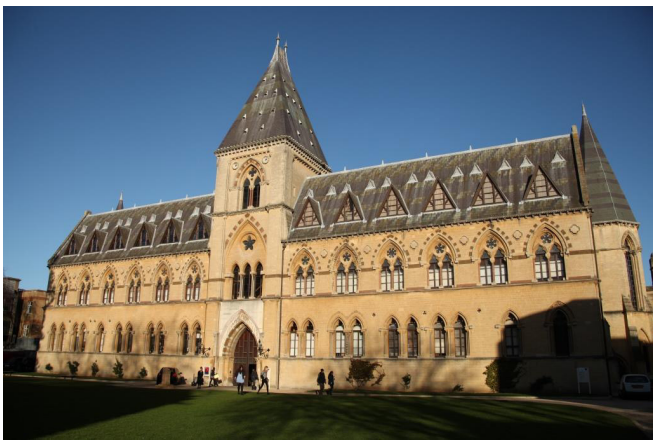
Into the Light:
The re-emergence of the Oxford University Museum of Natural History

by Bethany Palumbo

In January 2013, the Oxford University Museum of Natural History (OUMNH) closed its doors for 14 months, allowing for the long-awaited restoration of its original Victorian roof. The 150-year-old glass tiles were dirty and discoloured, and rainwater regularly leaked onto the displays below. The project was also the start of big changes for the Museum. The closure not only allowed for the permanent displays to be cleaned and conserved, but also presented an opportunity to build an effective media presence through the use of social media.

The OUMNH - formerly called the University Museum - opened in 1860 and was designed by the famous Irish architectural team, Deane and Woodward, who won the contract in a contest held by the Museum Delegates in 1854. One of the few stipulations of this contest was that the design must feature a glass and iron roof to cover the court. The design of the roof was innovative for the time, with cast iron pillars supporting the weight of the glass, and wrought iron décor focussing on the shapes and forms of natural history specimens.

Over the past 150 years, dirt and residue has built up on the surface of the glass, ruining the visual aesthetics of the building, while cracks in the glass resulted in multiple leaks. To restore the roof and spruce up the gallery, the University invested £2 million for repairs and new lighting, working with the construction company, Beard, and heritage architects Purcell, as well as the University's Estates Services. More than 8,500 glass tiles were individually removed, cleaned and sealed with a mastic silicone. Where necessary, replacement glass tiles were handmade to match the Victorian originals. The result is a beautiful, radiant space, with the new LED lighting in the court allowing the Museum to host events into the night.



The Oxford University Museum of Natural History on Parks Road houses the University's scientific collections, including life and earth materials and is open to the public. Image credit: OUMNH.

Neil Hyatt, the project manager for Estates Services, reflects fondly on 14 months of hard work:

> *"Whilst the project has been a complex one – the logistics of scaffolding such a huge space; keeping the interior protected from the heavens opening when the glass was out; the sequence of removal, cleaning and replacement; and ongoing Museum conservation work in the same space – it has been a pleasure and delight to work on. Having become dull and lifeless as the glass became progressively dirtier and more obscured, the roof now shines with a radiance not seen since it was first constructed."*

The closure also facilitated additional projects that would have been difficult to complete during normal opening hours. Construction scaffolding allowed staff to complete successful conservation work on a number of whale skeletons, which were treated for the first time in over 100 years.

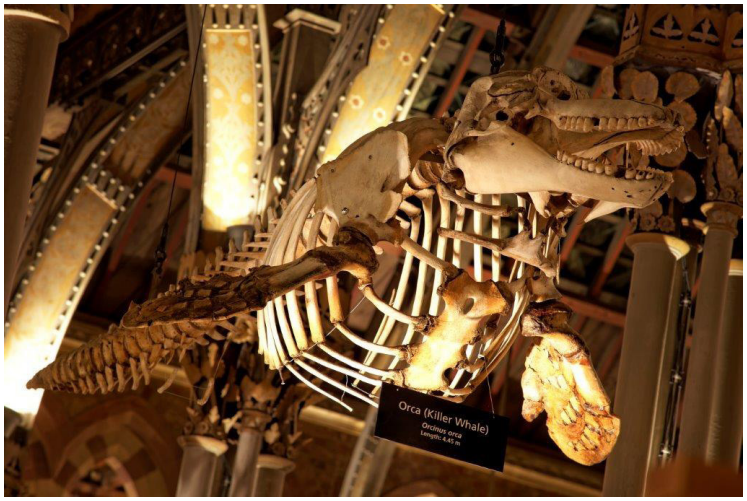
The whales were in poor condition. Foremost was the build-up of dust and dirt sticking to a dense layer of natural oils, which had been secreted and oxidised over the decades, forming a thick, discoloured coating. The Museum environment was also detrimental to the specimens, with continuous light and UV exposure weakening the matrix of the bone material, resulting in warping and cracking. The original copper and iron wiring used to articulate the whales had also corroded, weakening the surrounding bone.

With only a six-month period in which to complete the treatment, the Museum conservators prioritised treatment to include cleaning and stabilisation. Cleaning consisted of a vigorous vacuuming followed by the application of dilute ammonium hydroxide to remove the natural oils by converting them into soap through the process of saponification. Ammonia was selected over detergents or other cleaning products as it completely evaporates, leaving behind no residues.

Stabilising the specimens comprised two parts: consolidation and re-articulation. Areas of bone that were visibly weak were treated with an adhesive commonly used in palaeontological conservation. This provided additional strength to the bone matrix. Old wires were removed where appropriate and replaced with stainless steel, which is able to withstand the fluctuating environment of the roof space. This re-articulation also allowed the conservators to correct the anatomy of the specimens, which in some parts was not scientifically accurate. With the treatment completed, the specimens were hoisted back into the roof space in new positions, offering better views from the upper level of the Museum.

The original museum roof, circa 1890. Image credit: OUMNH.

Whilst the Museum was closed, a huge effort was undertaken to keep its activities publicised through the use of social media. This included the creation of the blog Darkened not Dormant and daily updates from the new Twitter feed @morethanadodo. Used to promote the internal activities hidden under the scaffolding, as well as outreach activities such as the Goes to Town trail in Oxford city centre, social media have proven to be effective channels of communication for the Museum. The results are impressive, with around 3,600 followers on Twitter and over 31,000 views of the blog to date.



Completed restoration of a killer whale skeleton. Image credit: OUMNH.

Scott Billings, communications officer for the Museum, describes the thinking behind this online activity:

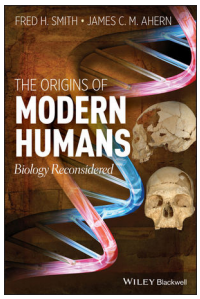
> *"Taking the Museum onto social media presented a very good opportunity to think carefully about how we wanted to communicate with different people and about what we had to say. Using platforms like WordPress for our blogs and Twitter for shorter messages and interactions has allowed us to reveal some of the hidden work of the Museum, as well as share interesting photographs and facts about the specimens and the building itself. Despite launching while the building was closed, or perhaps thanks to this, social media channels are now an important aspect of how we represent the Museum and engage with people far and wide, extending access to our collections."*

The Oxford University Museum of Natural History re-opened on 15 February 2014, with a dawntildusk event entitled 'Into the Light'. With live music and roving touchable specimens, the opening day boasted a record attendance of approximately 5,300 visitors. Without a doubt, it's good to have the Museum vibrant and bustling once more.

Whale project blog: www.onceinawhale.com
Closure blog: www.darkenednotdormant.wordpress.com
Main Museum blog: www.morethanadodo.com

Bethany Palumbo is the Conservator for Life Collections at the Oxford University Museum of Natural History. Her research concentrates the conservation and preservation of taxidermy and skeletal materials, preventive conservation and integrated pest management.

BOOK REVIEW



The Origins of Modern Humans: Biology Reconsidered

Edited by Fred H. Smith and James C. M. Ahern

ISBN: 978-0-470-89409-5, Wiley-Blackwell (2013)
Hardcover, 480 pages, £86.95

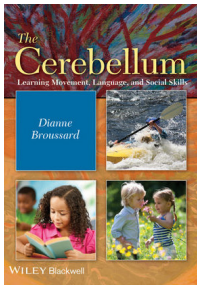
Reviewed by Dr Christian Eichinger

Where do we come from? What are the characteristics of our recent history as human beings? And what were the reasons for the incredible expansion of our modern human ancestors out of Africa? Informative and thought-provoking, *The Origins of Modern Humans: Biology Reconsidered* is an ideal read for students and professionals in human evolution and palaeoanthropology, as well as anyone seeking up-to-date answers from researchers at the forefront of the field.

Approximately 50,000 years ago, history witnessed a global expansion of modern humans out of Africa, their settlement across the globe and their encounters with the Neanderthal hominids. Due to the existence of several competing theories attempting to reconstruct these remarkable events, further genetic and palaeoanthropological evidence is required. This book provides comprehensive expert knowledge on the key discoveries made in the past 25 years.

A highlight is the introduction *Thoughts on Modern Human Origins: From 1984 to 2012* by the editors. This section tactfully reconciles major hypotheses of human evolution introduced in the previous 1984 volume with the current, most widely accepted theories, incorporating the latest information in palaeontological, genetic and developmental biology. The book then takes the reader on a journey. Starting from Africa, the "cradle of modern people", the 12 chapters follow the movement of modern humans into Europe and the rest of the world. The authors follow a regional approach, covering Africa, Asia, Europe, East Asia and Australasia, to discuss the collection of hominid fossil material in each region during the Pleistocene epoch.

Being built up of a collection of peer-reviewed scholarly pieces gives this work a distinctive touch. Also, rather than being a second edition *per se*, the book explicitly addresses novel analytical methodologies and features new fossil, dating and molecular evidences. This further includes inferences based on comparison of the genomes of modern humans with that of Neanderthals and how these call for re-evaluation of recent hypotheses. Overall, this update to the 1984 award-winning classic, *The Origins of Modern Humans: A World Survey of the Fossil Evidence*, is a long-awaited must-read for students and academics in the field, but is also capable of capturing the imagination of a lay reader with some interest in the topic.



The Cerebellum: Learning Movement, Language and Social Skills

Dianne M. Broussard

ISBN: 978-1-118-12563-2, Wiley-Blackwell (2013)
Hardcover, 240 pages, £86.95

Reviewed by Dr Ruth Faram

The cerebellum has long been considered an inferior 'little brain', sitting below the complex cerebral hemispheres. Broussard's eloquently written book, *The Cerebellum: Learning Movement, Language and Social Skills*, attempts to abolish the myopic view that the cerebellum is solely implicated in motor tasks, stating the case for other physiological functions to which the cerebellum can, in part, be attributed.

At first glance, Broussard's book may be technically challenging for anyone lacking the basics in neuroanatomy. Written with neuroscientists in mind, Section I offers a detailed account of the cellular structure, architecture and electrophysiological properties of neurons within the cerebellum. The general emphasis is that the cerebellum is not a linear system, but is composed of multiple systems that interact with themselves and other brain regions, permitting a large capacity for learning and plasticity. Although technical, the majority of neuroscientific terminology is explained throughout, increasing accessibility.

In Section II, Broussard describes the multiple roles of cerebellar plasticity and their implication in memory, motor learning, vision, orientation and proprioception. Having explained the intricate processes of synaptic changes and cell firing patterns in the cerebellum, Broussard introduces Section III. This discusses the relationships between ocular function and coordination, vestibular function and proprioception, complete with clinical examples of cerebellar dysfunction, which have shed light on the role of the cerebellum.

Section IV, the book's apex, focusses on human cognitive function. Introducing the hypothesis that humans evolved with expanded cerebellar posterior lobes and ventral dentate nuclei, Broussard discusses the evidence for the cerebellum contributing to higher functions, using examples of modern day brain imaging and clinical data. Acknowledging the infancy of this field, Broussard's descriptions are compelling enough to capture the interest of any neuroscientist.

The ultimate purpose of this book is to introduce the reader to the modern hypothesis that the cerebellum is finely tuned with higher brain functions. Broussard does this in a concise manner, though this perhaps loses impact in being restricted to the smallest, final section of the book. It is hopeful, as Broussard comments, that "in the next few years considerably more will be learned about the functions of the cerebellum", so that a later edition might contain a more detailed section on the higher cognitive functions of the cerebellum.

BOOK REVIEW

Cancer Cell Signalling

Edited by Amanda Harvey

ISBN: 978-1-119-96757-6, Wiley-Blackwell (2013)
Paperback, 228 pages, £37.50

Reviewed by Radhika Agarwal

Since the inception of the cell signalling field with the discovery of insulin about 90 years ago, the interest in communications within and between cells has compounded into a wealth of knowledge about specific signalling pathways. While individual characterisation of these pathways and their components is important, there is also much cross-talk between pathways. This is especially true with cancer cells. *Cancer Cell Signalling* is a well-structured "master-review" which follows this 'individual first, cross-talk second' consideration of the main signalling pathways implicated in tumour development and disease proliferation.

Cancer Cell Signalling is less of a beginner's primer and more a compilation of current knowledge regarding the major signalling pathways in cancer biology. Fourteen contributors make this book a rigorous and thorough review of the subject. The first eight chapters individually cover EGF, IGF, TGF-β, Wnt, mTOR, c-Met, VEGF and progesterone receptor signalling, while a final chapter is devoted to cross-talk. Though the chapters bring us to the cutting-edge of each field, the reader is not lost in complexity thanks to the homogeneous organization of the chapters. Each chapter begins with an introduction and historical timeline, followed by a presentation of the details of signalling using clear coloured figures. Finally, applications are summarised with regard to treatment strategies, including a discussion of chemotherapeutics that interfere with specific components of each signalling pathway and perspectives for the design of novel drugs and inhibitors.

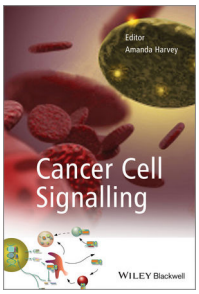
The elegant structure of this book lends itself to many audiences. It is a must-read for the beginning PhD student in cancer biology, whereas the more experienced researcher might use the book as a fairly comprehensive refresher course and reference guide. Of special note for those seeking inspiration in the field of drug design, the sections on therapeutic strategies that follow each pathway discussion would prove immensely useful. The book is also sufficiently understandable to be consulted by advanced undergraduate students seeking details about a specific signalling pathway for a class or tutorial. The companion website allows lecturers to download figures and tables free of charge. The low price of the paperback version, and the high quality and clarity of the text, make this a must-buy for any library or cancer lab.

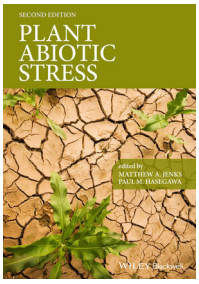
Plant Abiotic Stress (2nd Edition)

Edited by Matthew A Jenks and Paul M Hasegawa

ISBN: 978-1-118-41217-6, Wiley-Blackwell (2014)
Hardback, 336 pages, £133

Reviewed by Jessica Beevers





Plant Abiotic Stress is a compilation of chapters detailing current research into non-biological stress factors that affect plant growth and development. The first seven chapters each discuss a separate abiotic stress factor including drought, flooding, temperature, salinity, and toxic metals. The final three chapters deal with epigenetic regulation of abiotic stress, the genomics of abiotic stress tolerance, and genetic mapping using quantitative trait loci or association studies to aid breeding for crop improvement.

The book is a carefully crafted summary of the current understanding of the biochemistry that drives plant responses to abiotic stress. Each stress is covered in as much detail as is possible without devoting an entire book to the individual topics. A full bibliography follows each chapter, providing a useful reference for students new to plant sciences. Additionally, the book includes clear figures in black and white, and a central section with colour versions of some of the figures.

There are two minor weaknesses in the text: First, the proofreading could have been more thoroughly executed. The careful reader will find a couple of marginally irritating grammatical errors in nearly every chapter. Additionally, some topics are introduced repeatedly and discussed in very similar terms by several of the chapter authors. This can be tiring, although it does serve to highlight the importance of certain concepts. These issues are, however, both easily overlooked and do not pose serious difficulties.

Overall, this text provides a useful launch pad for students who have not previously studied plant abiotic stresses and need some initial direction in their studies. For those who are more familiar with the topic, the book serves as a useful reference that conveniently compiles a vast body of knowledge into a single place for ease of use.

5' with... Prof Jane Langdale

Jane Langdale is a Professor of Plant Sciences and Senior Research Fellow at The Queen's College. She studied Applied Biology at the University of Bath before undertaking a PhD in Human Genetics at the University of London. After her doctorate, she did post-doctoral research in Plant Sciences at Yale University, focusing on the molecular and genetic basis of plant development. Since 1990, she has led her own research group in the Department of Plant Sciences at Oxford, most recently studying the evolutionary basis of plant development.

Interviewed by Stephanie Kapsetaki

When did you first decide you wanted to be a career scientist?
I did Biology as an undergraduate and for a PhD because I enjoyed science and appreciated the scientific approach. I love solving problems which is part of what scientists do: set a hypothesis, design and carry out experiments to test it, and then use the results to try to solve the problem. But it probably wasn't until I was in my late twenties that I thought I could do it as a serious career and run my own research group.

Why did you choose to study plants?
I did my PhD in human genetics. One of the reasons I switched was because if you do human genetics you have to work with existing populations – you can't exactly set up your own crosses! For my postdoc I wanted to work with a more genetically tractable, but still multicellular, system. At the time the choice was either *Drosophila* or plants. I chose plants and spent many years doing maize genetics, which I loved. There is nothing like spending three weeks of the summer in the middle of two acres of corn to really understand your experimental organism.

If you weren't a scientist you would be…
Probably a property developer. I'd buy run-down houses and renovate them. I enjoy bringing order to chaos and I am both imaginative and practical when it comes to such things.

If you are not in the lab you are…
Hanging out with my dogs in the garden. I have a big garden and it needs a lot of attention.

What was your worst disaster in the lab?
I dropped a bottle of mercaptoethanol once in front of the fume hood, not *in* the fume hood. That wasn't good. I also spilt phenol over my legs when I had shorts on. That wasn't good either!

What has been the most memorable finding in your career so far?
The most satisfying is one that we are currently exploiting in an attempt to engineer leaf anatomy in plants. Plant species are described as C_3 or C_4 based on the type of photosynthetic cycle that they use. In general, C_4 crops, such as maize and sorghum, are more productive than C_3 crops, such as rice and wheat. During my postdoc I discovered that some of the leaves within the C_4 maize plant actually develop in the same way as C_3 leaves. With the new genomics-type technologies, we have recently been able to compare gene expression patterns in C_4 and C_3 leaves of individual maize plants and now have a handle on the genes that might be regulating the distinctive leaf anatomy that develops in C_4 plants. We are using this knowledge to contribute to a multinational collaborative effort that aims to increase crop yields in the C_3 plant rice by engineering C_4 traits into it.

Do you have a favourite classical experiment?
I think Barbara McClintock's work on transposable elements is my favourite.

What is the best advice you have ever received?
Do what you enjoy doing!

How do you imagine biological research will change over the next 20 years?
I think it will be a lot more computer-based. A lot more of designing and engineering experiments *in silico* and a lot less time at the bench actually doing the experiments.

Write for *Phenotype*?

- The deadline for article submission is Friday of 8th week, 20 June 2014
- We accept articles on any aspect of biological sciences research, books or science education
- Articles can be either 650 or 1300 words

If interested, please get in touch: oubs@bioch.ox.ac.uk.

Work for *Phenotype*?

If you'd like to get involved in editing, production or management of *Phenotype*, please get in touch: oubs@bioch.ox.ac.uk.

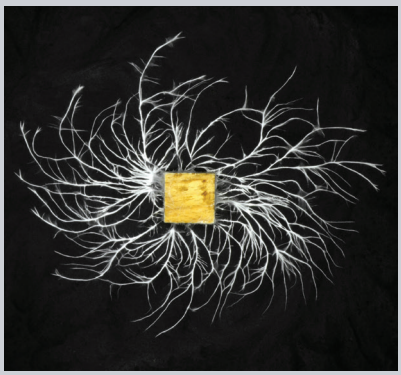
SNAPSHOT Research Image Competition

This issue's winner is...

Dr Luke Heaton

Dr Luke Heaton is a post-doctoral research assistant in the Fricker group in the Department of Plant Sciences.

The winning image portrays the saprophytic, cord-forming fungus *Phanerochaete velutina*, which is busy digesting the central 2 cm x 2 cm wood block and exploring its microcosm in search of more things to digest.



Luke obtained his Bachelor of Science in Mathematics from the University of Edinburgh. He then continued to the University of Oxford to study for a Masters of Science in Mathematics and Computer Science. After a break from science, during which he completed a BA in Architecture, he returned to Oxford to complete his DPhil in the Physics Department as a member of the Life Sciences Interface Doctoral Training Centre (DTC). Luke is currently working in Dr Mark Fricker's laboratory, constructing mathematical models relating to fungi.

There are millions of fungal species in the ecologically crucial fungal kingdom. In order to survive, many fungi form networks that forage through substrates for scarce but vital resources, such as phosphate and nitrogen, to then coordinate the transport and distribution of these nutrients. Despite fungi being a crucial soil component, our understanding of these organisms is lacking. The Fricker group employs a combination of experimental techniques and mathematical models to answer the following questions: How are local patches of useful materials analysed, moved, and integrated to enable a fungal network to grow as a coherent whole? What are the mechanisms involved in the transport process? What is the logic behind the development of these fungal networks?

The Fricker group uses scintillation screens and photon counting cameras to trace the movement of radiolabelled materials through a fungal network. Image processing techniques can then be used to convert photographs of these networks into digitised representations. Results from Luke's doctoral research suggest that a combination of turgor pressure and localised asymmetric growth induces bulk flows of material across the network, and that these flows may allow for efficient foraging behaviour. Given the heterogeneous distribution of resources within the soil, and the energetic demands imposed by maintenance, growth, transport and the production of digestive enzymes, Luke's current research proposes that observed patterns of fungal growth may be the result of optimal foraging strategies.

In addition to fungal physiology, Luke is interested in bioenergetics, morphogenesis, and the complex interactions between soil, fungi and plants. He also enjoys making art and writing about the history and philosophy of mathematics. He has signed a contract with the publishers Little Brown and his popular science book, *The Language of Patterns, or what is Mathematics and where does it come from*, is due to be published in early 2015.

OXFORD UNIVERSITY PRESS

SNAPSHOT
Research Image Competition

Win a £50 book voucher kindly provided by Oxford University Press!

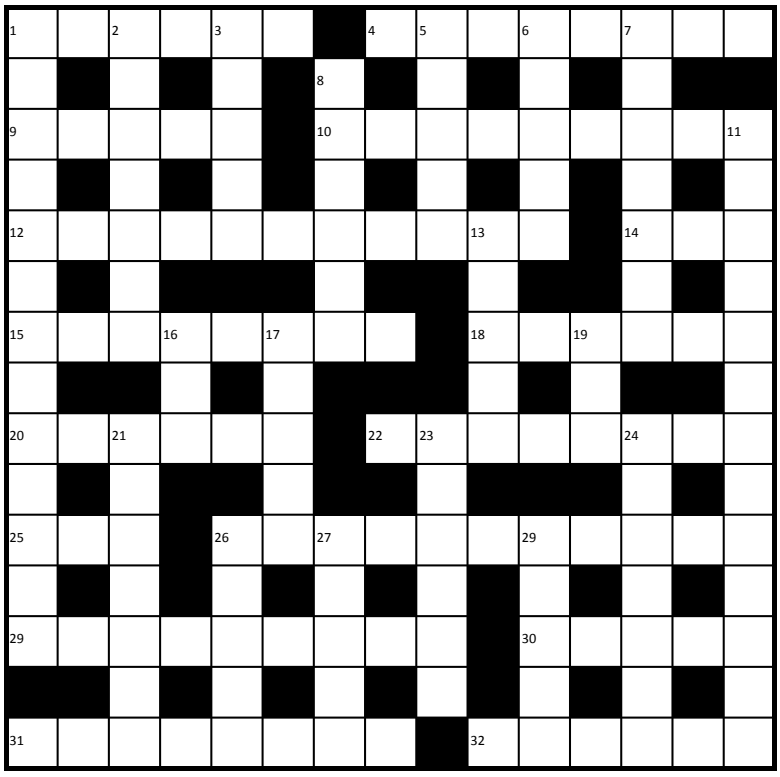
Do you have an image from, or inspired by your research? Why not enter it in SNAPSHOT?
We are now accepting entries for pictures to be featured on the cover of *Phenotype* Michaelmas 2014.
To enter, send images to oubs@bioch.ox.ac.uk with a brief description (maximum 100 words).
Please get permission from your supervisor before sending any images.
There is no limit to the number of entries per person.

The deadline for the competition is Friday of 8th week, 20 June 2014.

PHENOTYPE crossword

It would seem that our resident cryptographer, *Fish*, was just too "Wiley" for you last issue as no one has won our book prize. Check out the answers at the bottom of the page. *Fish* challenges *Phenotype* readers to this latest cryptic crossword on the theme of plant science. Can you crack it this time?

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 Friday of 8th week, 20 June 2014, will be entered into the prize draw.



Who will win the prize from this issue?

The winner can choose one of the four books reviewed in this issue, generously provided by Wiley-Blackwell.

WILEY-BLACKWELL

Across

1 Males guided back by one who worked with 14 acrosses (6)
4 Police make one airhead eat 100 Brussels sprouts, perhaps (8)
9 Cut signalling pathway (5)
10 Cultivated *Angelicas* produce a painkiller (9)
12 Those endlessly achy and dolorous at heart tell Phil to fetch them green pigment (11)
14 Exercise a legume (3)
15 Nut carrying seed? (8)
18 Place diamond, say, on 10 of hearts (6)
20 Centre for aligning wood fibre (6)
22 First one of the Sopranos has my back at meetings (8)
25 Drive out every second and perish (3)
26 Plot scholar's failure in containing 12 across (11)
29 Clark follows the Blue Man Group? It's passable (9)
30 Impale small fruit (5)
31 Straddle favourite horse (8)
32 Curtail silicon content of 14 across from Iran, once (6)

Down

1 No model tycoon grows a type of flowering plant (13)
2 Bugs with stingers? (7)
3 Anaesthetic knocks one out, or . . . (5)
5 . . . is prepared in 50 year bursts (5)
6 Immunoglobulin cross-links sulphur and interleukin - it's a sign! (5)
7 Measure taste and identify one that's weak (7)
8 Descend or ascend as part of Def Leppard concert (6)
11 Icy carbonate: a compound that may be found in a bloom? (13)
13 Trim end of column and mix to produce substitute (5)
16 Make leather brown (3)
17 Trick church out of its shell (5)
19 Spanish flower? (3)
21 Mourns vocally for shin protectors (7)
23 Two short men (one backwards, one forward) told tales (6)
24 The last word in good men - they carry pollen (7)
26 Soldier gets back into vehicle with rolled tobacco (5)
27 Threw a note out because it was protuberant (5)
28 Stick point into scalp (5)

Answers to the crossword from issue 17 | Hilary '13

Across: 1 colitis; 5 *calor*; 8 *rubor*; 9 rhodopsin; 11 sou; 12 castes; 13 nearest; 15 born; 16 strafes; 18 scry; 20 neonate; 21 maniac; 23 tea; 24 spaghetti; 25 *dolor*; 26 *tumor*; 27 nucleus

Down: 1 cardinal signs; 2 laboratory; 3 tarsiers; 4 structuralists; 5 chorea; 6 (harm-)less; 7 loss of function; 10 not on the cards; 14 fontanelle; 17 sporadic; 19 rasher; 22 harm(-less)