OpenPlant

Annual Report 2019

BBSRC-EPSRC Synthetic Biology Research Centre
UK Synthetic Biology for Growth programme

OPENPLANT ANNUAL REPORT 2019

EXECUTIVE SUMMARY

Progress in Year 5

In our last year of operation (Sept 2018-2019), we have continued to make progress in development of: i) foundational technologies for standardised DNA assembly in plants, and simple systems for plant synthetic biology, ii) trait development with improved capacity for metabolic analysis and reprogramming of metabolism in plants, and (iii) activities for outreach and responsible innovation. Our work has resulted in numerous new publications, scientific presentations, and novel outreach and Biomaker activities which are listed in the attached report. A summary of the novel aspects per workpackage can be found below:

Workpackage A: Simple Plant Chassis, Tools and Gene Delivery

1. Optimisation of the Marchantia spore transformation procedure has continued with a focus on the facile production of sterile spores.
2. We performed optimisation experiments and chosen mTurquoise-2, eGFP, mVenus, and mScarlet-1 as the key members of the fluorescent protein gene family for multi-spectral imaging experiments in Marchantia.
3. Work continues on the genome-wide collection of putative promoters from sequences proximal to Marchantia transcription factor genes, with the construction of Level 1 and Level 2 gene expression vectors.
4. Marchantia genome sequence data has been revised in the last year (ver 4.0 and 5.0) and shared with us by Dr. Fred Berger and colleagues.
5. DNAs will be distributed through Addgene, and we await implementation of the OpenMTA in their distribution systems. In the meantime, we have distributed material directly, using the implementation of the OpenMTA at the University of Cambridge.
6. We have recently published our work on DNA methylation in Marchantia (Aguillar-Cruz et al., 2019), and the Loop Assembly method (Pollak et al., 2019).

Workpackage B: DNA Assembly

7. A previously identified electrically regulated promoter element from cyanobacteria was also found to be functional in *E. coli* as a repressor, although we are unsure of the identity of the *trans-acting* factor in this heterologous system.
8. We have published our work on the development of biophotovoltaic systems (Rowden et al., 2018; Wey et al., 2019), and the genomic and transcriptomic analysis of the peridinin dinoflagellate *Amphidinium carterae* plastid (Dorell et al., 2019).

Workpackage C: New mechanisms for Regulation of Gene Expression

9. To be able to build and analyse novel riboswitches in *Chlamydomonas* in a high-throughput manner, we used the *Chlamydomonas* Golden-Gate MoClo kit to construct a series of riboswitch-regulated fluorescent reporter genes. Use of fluorescent proteins, as C-terminal tags of transgenes or as reporters of promoter activity, was only possible until recently and is now a widely used strategy in our lab for both *Chlamydomonas* and *Pheaodactylum* alga models.
10. We have demonstrated the utility of the thiamine riboswitch in *Chlamydomonas* for metabolic engineering, by placing the casbene synthase enzyme under regulatory control of the riboswitch, which allowed tuneable production of casbene diterpenoid dependent on exogenous thiamine concentrations.
11. We have published our work on miRNA-mediated regulation of gene expression in *Chlamydomonas* (Chung et al., 2019; Navarro et al., 2019).

Workpackage D: Genome Engineering

12. A suite of variants of Cas proteins from different bacterial species was systematically compared resulting in an expanded standardized toolkit for genome engineering in plants (Raitskin et al, 2019, PloSOne). Methods for protoplast delivery developed in this study were also applied to potato (Workpackage G) which resulted in publication (Tuncel et al., 2019, Plant Biotechnology Journal).

Workpackage E: Digital Tools

13. We have developed imaging and image-processing methods, which allow simultaneous tracking of tissue kinetics and dynamics of gene expression.
14. We have, in a collaborative effort with Monica Dayao, employed deep learning methods to create a reliable binary segmentation mask for labelling pixels in microscopy images as cell, or not cell.

Workpackage F: Modules for engineering photosynthesis in leaf metabolism

15. We have constructed a series of Loop assembly vectors suitable for chloroplast transformation. These enable rapid construction of new genes that are suitable for delivery to the Marchantia chloroplast genome.
16. We have published our work on transcription factor binding during C4 photosynthesis (Borba et al., 2018; Burgess et al., 2019).

Workpackage G: Carbohydrate Engineering

17. We have expanded the tools for engineering dicot secondary cell walls, generating novel xylan structures in Arabidopsis.
18. We are currently exploring artificial, *in vitro* metabolic cycles, driving production of glucose-based oligosaccharides from cheap and readily available sucrose by using sucrose phosphorylase and glucan phosphorylases. This has resulted in the identification of two new families of carbohydrate-active enzymes from Euglena: a new family GH149 (Kuhaudomlarp et al., 2018) and GH161 (Kuhaudomlarp et al., 2019).
19. Genome editing tools and technologies developed in the Patron lab (Workpackage D) were applied to generate potatoes that putatively contain digestion-resistant starches with potential nutritional benefits (Tuncel et al 2019). The impact on the gut microbiome of starches produced in this study will shortly be investigated by the Warren Lab at the Quadram institute, using mice with humanised gut microbiomes.
20. We are evaluating the potential of cereal endosperm as platform for cytosolic expression of enzymes able to synthesise glucans. The work was in part funded by BBSRC grant BB/K006517/1, and is in preparation for publication.

Workpackage H: Tools for Engineering Plant Natural Products

21. We have exploited available genome sequence resources to carry out a large-scale investigation of triterpene biosynthesis across the Brassicaceae (Liu et al., submitted). Our results indicate that plant genomes are remarkably plastic, and that dynamic Genome Neighbourhoods (GNs) generate new biosynthetic pathways by shuffling the genes encoding a core palette of triterpene diversifying enzymes, presumably in response to strong environmental selection pressure.
22. We have investigated how plant biosynthetic gene clusters (BGCs) are maintained and diversified at the within species level, resulting in the first genetic evidence supporting co-inheritance of metabolic gene clustering in plants.

23. We have developed a systematic approach to mining the *Reaxys Natural Product Databases* (RNPD), allowing the surveying of natural oxidation diversity of triterpene scaffolds.
24. We have elucidated the function of a suite of new triterpene biosynthetic enzymes, including enzymes responsible for biosynthesis of insecticidal limonoids (Hodgson *et al.* PNAS), antimicrobial defense compounds (Leveau *et al.* New Phytol), (Louveau *et al.* Plant Cell), (Orme *et al.* PNAS, submitted), and enzymes comprising a novel gene cluster in the Brassicaceae (Liu *et al*., submitted).
25. We have published a highlight article (Stephenson *et al.* Natural Product Reports) on novel triterpene stereochemistry that we have discovered in an earlier collaborative project. This stereochemistry represents a potentially transformative discovery in triterpene biosynthesis, breaking a dogmatic dichotomy which has existed for over 60 years.

Workpackage I: N2 Fixation

26. We have published our work on host responses to oomycete infection in an early-divergent land plant lineage (Carella et al., 2019; Carella et al., 2018).
27. Using the *M. paleacea* genome as foundation, we have analysed the conservation of the symbiosis signaling pathway and related genes across a wide range of species. This has revealed invariant conservation of the Sym pathway in all species accommodating intracellular symbioses, including *M. paleacea*, but not *M. polymorpha.* Such conservation is independent of the symbionts and present in many diverse intracellular symbioses
28. We have demonstrated through biochemical approaches and transcomplementation that the Sym pathway components of *M. paleacea* are functionally conserved with their orthologs in legumes.
29. After challenges establishing Cas9 mutagenesis in *M. palecea*, we have finally been able to generate knockout mutants in this plant. The first genes mutated are *NSP1* and *NSP2*.
30. Promoter-GUS fusion analyses in *M. paleacea* have revealed reporters activated at different stages of arbuscular mycorrhizal colonisation.
31. We have submitted our work on *M. paleacea* for publication, currently under review and have placed the paper on Biorxiv..

Workpackage J: Virus-based systems for bioproduction

32. The pHREAC vector has proved to an effective way of expressing RNAs which, unlike RNA produced by pEAQ vector, have no residual ability to be replicated and thus cannot be packaged within CPMV particles.
33. It has been shown recently that the presence of replicating RNA greatly facilitates the assembly of filamentous viruses. This will facilitate structural analysis of such particles and their use for the display of antigenic sequences.
34. Research on the use of the hypertrans system for the expression of proteins in micro-algae is currently underway in collaboration with Sogang University, S. Korea.
35. Our work has resulted in various new publications; this includes our work on optimization of transient expression systems (Peyret et al., 2019), and enhanced production of, and structural insight in virus-like particles (Byrne et al., 2019).

Workpackage K: OpenPlant Fund for interdisciplinary exchange

36. In this last year of the OpenPlant grant, we have combined the OpenPlant Fund call with the Biomaker Challenge, and offered teams an initial £750 plus a hardware package worth £250. After the first stage, teams could apply for an additional £2000 follow-on funding. We have had 27 diverse teams, of which most of them applied for and received follow-on funding.

37. We made *ad hoc* OpenPlant Fund awards to outstanding projects, including a molecular biology workshop in Benin, a speed breeding workshop in Kenya.

Workpackage L: OpenPlant Forum: responsible innovation

38. The 2019 OpenPlant Forum was themed “Smart Design for the Future Bioeconomy” and took place in Cambridge from 19-31 July.
39. In this last year we have delivered new OpenPlant-themed workshops in primary schools, designed and delivered by research scientists in collaboration with SAW, influencing the educational approach to these topics.
40. In the past year, we have also engaged with the public by delivering an inspiring interactive exhibit at the London Lates (“Year of the Engineer”, Oct 2018).
41. SAW developed a training workshop to enable dissemination and to share best practice with other research centres. In November 2018, SAW ran a workshop session for “Talking Plants”, the annual conference of the Botanic Gardens Education Network, hosted at Cambridge Botanic Gardens. Garden staff from across the UK’s network came together to explore ways of bringing plant science to a wider audience.
42. We are preparing to run the next Global Gardens workshop at Cambridge Botanic Gardens in October 2019 as part of the city-wide Festival of Ideas.
43. We are preparing a ‘Build your own DNA Dave’ workshop for schools, helping schools to build a machine that explains the processes of transcription and translation in a fun and interactive way.

Workpackage M: Governance and Management

44. OpenLabTools activities are delivered through the Biomaker Challenge. The Biomaker Challenge was awarded £80K from GCRF for implementation of the programme in Ghana, Egypt. South Africa and Ethiopia to stimulate training and local capacity building and innovation.

RESEARCH PROGRAMME

Workpackage A: Simple Plant Chassis, Tools and Gene Delivery

Relationship to other projects/themes

The liverworts (or Marchantiophyta) are descendants of the earliest terrestrial plants. The group is characterised by morphological simplicity, and this is matched by simple underlying genome structures. Many lower plants, including liverworts, demonstrate a striking tolerance of extreme stresses, a trait that would be valuable in a production system. Liverworts have been a largely neglected area of plant biology, but show promise as new experimental systems after recent developments in transformation methods and genome characterisation. *Marchantia polymorpha*is the best characterised liverwort plant. It is a common weed, and can grow quickly and resiliently. The relative simplicity of genetic networks in Marchantia, combined with the growing set of genetic manipulation, culture and microscopy techniques, are set to make this primitive plant a major new system for analysis and engineering. We aim to establish Marchantia as a testbed for plant synthetic biology, which will provide a prototype for other OpenPlant initiatives in higher plants.

Investigators

Jim Haseloff (15 days); Giles Oldroyd (1 day); Jim Ajioka (4 days); Pietro Cicuta (6 days); Lisa Hall (2 days)

Staff Employed

Past:

Tim Rudge (PDRA; Cicuta lab). Aug 2015 - Feb 2016.
Tom Meany (PDRA; Haseloff and Hall labs). Oct 2015 - Jan 2017.
Fernan Federici (PDRA, Haseloff lab). July-Dec 2015; July-Dec 2016.
Linda Silvestri (Research technician, Haseloff lab). February 2016-Sept 2019.

Current:

Susana Sauret-Gueto (Research Manager; Haseloff lab). Started October 2015.
Eftychios Frangedakis (PDRA, Haseloff lab) Started April 2017
Alan Marron, (Research technician, Haseloff lab) Started August 2019.
Marta Tomaselli (OpenPlant PhD student). Started April 2017

Partners

Bernardo Pollak; Christian Boehm; Mihails Delmans, Marius Rebmann

Aims

The aim of this workpackage is to exploit the extraordinary experimental properties of Marchantia, and produce systematic collections of (i) experimental protocols and (ii) shared DNA parts. This will include a comprehensive collection of promoters, selection markers, and fluorescent and biopigment reporter genes. In addition, (iii) we will produce and distribute Marchantia lines with integrated cell fate markers in order to track physiological and morphogenetic changes. We will distribute information using laboratory-based web sites and specialised DNA registries, and distribute DNAs and plant material via NASC. The same methods for public distribution will be used widely in other OpenPlant workpackages.

Milestones
A1: Establishment of laboratory for automated DNA assembly, measurement, and quantitative imaging.
Deliverable: Commissioning of the OpenPlant laboratory (month 9, Haseloff).

A2: Establishment of microfluidic platforms for high throughput single cell imaging.
Deliverable: Production of microfluidic devices for culture, sensing and time-lapse observations (month 24, Cicuta, Hall).

A3: Establishment of Marchantia spore transformation system.
Deliverable: Validation of Marchantia spore transformation using GFP expression (month 24, Haseloff, Oldroyd, Schornack, Osbourn, Smith).

A4: Distribution of new Marchantia vectors for quantitative imaging.
Deliverable: Public release* of DNA vectors with ratiometric fluorescent markers (month 36, Haseloff).

A5: Distribution of a collection of Marchantia transcription factor promoters.
Deliverable: Public release* of a collection of synthetic promoter DNA parts (month 60, Haseloff, Harrison).

A6.1: Cell and tissue type transcriptomes.
Deliverable: Analysis and publication* of Marchantia tissue-specific transcriptome data (month 60, Haseloff, Harrison).

A6.2: Distribution of cell and tissue type specific expression vectors and lines.
Deliverable: Public release* of a collection of Marchantia promoter-GFP gene fusions with tissue-specific expression patterns (month 60, Haseloff, Harrison).

Progress (2018-2019)
A1-A2: Covered in previous reports.

A3: In addition to work described in previous reports: Optimisation of the Marchantia spore transformation procedure has continued with a focus on the facile production of sterile spores. Five litre Microbox containers (SacO2) and autoclaves Jiffy-7 peat disks are now routinely used for sterile growth of Marchantia plants throughout the life cycle. This allows the large-scale harvesting of sterile spores that can be stored and reliably used for nuclear and chloroplast transformation experiments. The closed culture method also minimises the potential for contamination of lines through transfer of sperm or spores between cultures.

A4-6: In addition to work described in previous reports: Dr. Sauret-Gueto (Haseloff Lab) has performed optimisation experiments and chosen mTurquoise-2, eGFP, mVenus, and mScarlet-1 as the key members of the fluorescent protein gene family for multi-spectral imaging experiments in Marchantia. All have been tested successfully in stably transformed plants. Coding sequences and genes have been packaged for open distribution.

Work continues on the genome-wide collection of putative promoters from sequences proximal to Marchantia transcription factor genes, with the construction of Level 1 and Level 2 gene expression vectors. So far, 74 transgenic lines have been produced and screened by fluorescence microscopy for gene expression. This has resulted in a high proportion of bright

and specifically labelled marker lines. These lines provide a unique and growing collection of novel cellular markers that can be visualised with unparalleled precision during development.

At this point, there is no shared facility for distribution of Marchantia germplasm. International exchange is severely hampered by the requirement for phytosanitary certificates with individual inspections of material and cumulatively expensive fees. We are discussing partnership between OpenPlant and the Nottingham Arabidopsis Stock Centre (NASC), to implement their system for maintenance and distribution of lines. We plan to apply for BBSRC BBRF funding to support this effort. This would create a badly needed hub for exchange of marker lines and mutants.

Marchantia genome sequence data has been revised in the last year (ver 4.0 and 5.0) and shared with us by Dr. Fred Berger and colleagues. This has enabled chromosome scale genome assemblies, and is allowing naming of genes by definitive genome location. Further the Haseloff lab has collected single-cell transcriptomic data from 4 day old germinating gemmae. Marius Rebmann has analysed the distribution of mRNA found in the sampled cells and classified the range of cell types found in these tissues. He and Marta Tomaselli are collecting new transcriptomic datasets to deconvolve dorsal and ventral and apical and distal cell transcripts, and analysing cell marker lines to validate these maps.

All lines, as well as the L0 parts and vectors, are being made freely available to the community under the OpenMTA license (launched with a published commentary in Nature Biotechnology 36:923–927, 2018). DNAs will be distributed through Addgene, and we await implementation of the OpenMTA in their distribution systems. In the meantime, we have distributed material directly, using the implementation of the OpenMTA at the University of Cambridge.

In addition, Alan Marron was hired on a short term contract to extend a GAL4-GFP enhancer trap screen in Marchantia that is highly productive. He is producing a range of exceptional marker lines that include specific labelling of new cells in the meristem, thallus margins, air chambers and oil cells.

Evidence of the quality of the research

2018-2019 Publications:

Aguilar-Cruz A, Grimanelli D, Haseloff J, Arteaga-Vázquez MA. *DNA methylation in Marchantia*. New Phytologist, 223: 575-581 (2019).

Pollak B, Cerda A, Delmans M, Álamos S, Moyano T, West A, Gutiérrez RA, Patron N, Federici F and J Haseloff. *Loop Assembly: a simple and open system for recursive fabrication of DNA circuits.* New Phytologist, 222: 628-640, (2019).

Kahl L, Molloy J, Patron N, Matthewman C, Haseloff J, Grewal D, Johnson R and D Endy, OpenMTA: *opening options for material transfer*. Nature Biotechnology, 36:923-927, (2018).

Other evidence of impact

Distribution of Cam-1 and Cam-2 Marchantia germplasm and plasmids.
Public access to MarpoDB website.

Invited presentations (Sept 2018-2019)

Plant Synthetic Biology, Bioengineering, and Biotechnology Conference, Cambridge, UK, plenary speaker (Haseloff).
Nature Plant of the Future Conference, New York, USA (13-14 Jun 2019), invited speaker (Haseloff).
Gordon Conference: Chloroplast Biotechnology, Ventura, USA (6-10 Jan 2019), invited speaker (Haseloff).
Banbury Meeting: Revolutionizing Agriculture with Synthetic Biology, CSHL, USA (2-5 Dec 2018), invited speaker and participant (Haseloff).
SC2.0 Synthetic Yeast Genome Meeting, Sydney, Australia (26-30 Nov 2018), invited speaker (Haseloff).
Robert Symons Memorial Lecture, Adelaide, Australia (8 Nov 2018), speaker (Haseloff).
AGTA meeting, Adelaide, Australia (4-7 November, 2018), invited speaker (Haseloff).
International Plant Systems Biology Meeting, Roscoff, France. (10-14 Sept 2018), invited speaker (Haseloff).

Workpackage B: DNA Assembly

Relationship to other projects/themes

Standards for DNA parts and assembly underpin the development of other technologies and platforms and therefore the outcomes of Workpackage B are relevant to other platforms and technologies, especially Workpackage D (Genome assembly).

The genome editing tools and large-scale gene assembly technologies developed in Workpackages B and D will be of direct benefit to all the trait-engineering work-packages (F, G, H, I and J). Specifically, vectors for chloroplast manipulation and methods to achieve homoplasty are being developed for use in Workpackage F and molecular tools for targeted genome editing are being developed for use in Workpackages G, H and I.

Investigators

Nicola Patron (5 days); Jim Haseloff (5 days); Jim Ajioka (4 days); Giles Oldroyd (0.5 days); James Locke (3 days); Christopher Howe (7 days)

Staff Employed

Past:
Douglas Griffith (PDRA, Locke lab). Started July 2015 - Ended November 2016
David Willey (PDRA, Ajioka lab). Started September 2015 - Ended March 2016
Orr Yarkoni (PDRA, Ajioka lab). Started May 2016 - Ended February 2018
Current:
Oleg Raitskin (PDRA; Patron lab). Started Jan 2015. Re-employed at EI Sep 2016
Susana Sauret-Gueto (Research Manager; Haseloff lab). Started Oct 2015
Eftychios Frangedakis (PDRA, Haseloff lab) Started April 2017
Philip Carella (PDRA, Schornack lab). Started Sep 2016
Bruno Martins (PDRA, Locke lab). Started Jan 2017
Stephen Rowden (PDRA, Howe lab). Started Sep 2017

Partners

Henderson lab, University of Cambridge
Uauy lab, John Innes Centre
Wendy Harwood, John Innes Centre

Aims

Hierarchical DNA assembly methods are a necessary part of genome construction and modification. These two workpackages aim to (i) set standards for plant synthetic biology; (ii) establish registries for sharing of plant-specific DNA parts; (iii) generate collections of plant DNA parts; and (iv) create standard tools for the engineering of plant genomes.

Milestones

B1: Simple, non-technical guide to installation of a DNA registry.
Deliverable: Online publication of a simple installation guide for use in OpenPlant laboratories (month 6, Haseloff).

B2: Installation of central database for sharing of published DNA details.
Deliverable: Installation of servers and publication of first records (month 24, Haseloff, Patron, Oldroyd).

B3.1: Characterised inducible cyanobacteria gene promoters.
Deliverable: Public release* of cyanobacterial DNA promoter part collection (month 30, Locke).

B3.2: A suite of biological parts that enable construction of synthetic circuits in Synechococcus elongata.

Deliverable: Public release* of S. elongata DNA circuit element collection (month 30, Locke).

B4: Parts for electrically regulated gene expression.
Deliverable: Public release* of voltage regulated cyanobacteria DNA parts (month 60, Howe).

B5: Low-cost license-free distribution of DNA parts.
Deliverable: Establishment of a badged OpenPlant part collection for DNA distribution (month 60, All)

Progress to date

B1-B3: Covered in previous report.

B4: The biophotovoltaic (BPV) system has the potential to form the basis of an innovative method for controlling the expression of biomolecules in modified micro-algae. Such a system would be of great scientific and commercial interest, and could be used for controlled expression of biopharmaceuticals, nutraceuticals and metabolic proteins. In the Howe lab, RNA-Seq analysis comparing electricity-generating biofilms to non-electricity generating biofilms of the cyanobacterium *Synechocystis* has allowed the identification of a number of putative electrically regulated promoters. Further, a particular cis-regulatory element was present in the promoter region in 6 of the 10 genes that showed the highest transcript levels and were also differentially expressed in the RNA-Seq data. This element is conserved in many other species of cyanobacteria opening up the possibility that it could be used as a universal BioBrick for synthetic biology in cyanobacterial species. The importance of the cis-element, and the minimal region required for regulation, is being ascertained through the construction of multiple promoter variants controlling a codon optimised mOrange2 using a modular cloning suite (based on the Plant MoClo Syntax) called Cyanogate that we developed with Dr. Alistair McCormick. To ensure the promoters being characterised are inducible in a wide range of light conditions the RNA-Seq data have been validated using qRT-PCR in a variety of conditions. Initial results characterising a 'super-strong' promoter have been very promising, and we can detect increased fluorescence from cells collected from electricity-generating biofilms compared to cells from non-electricity generating biofilms. However, the BPV system being utilised is not suitable for high-throughput screens and only allows for end-point analysis. Therefore, a more suitable BPV has been designed and constructed, and is currently being characterised. The new device has 12 separate but identical wells, excellent control of environmental conditions, and is compatible with the SpectraMax iD3 and iD5 monochromator plate readers, allowing time-point analysis of absorbance (growth) and the fluorescence (reporter) emitted from the biofilms. Using a simple assay as a proxy for electrogenic activity, we have also screened a set of mutant strains to identify which may be most useful as chassis organisms. We have also discovered that this *cis*-regulatory element is functional in *E. coli* as a repressor, although we are unsure of the identity of the *trans-acting* factor in this heterologous system.

B5: Covered in previous report.

Evidence of the quality of the research

Publications

Rowden S, Bombelli P and Howe C (2018) Design and study of bio-electrochemical system for biotechnological applications and metabolic investigation in Photosynthesis. Methods Mol Biol. 1770:335-346. doi: 10.1007/978-1-4939-7786-4_20.

Wey LT, Bombelli P, Chen X, Lawrence JM, Rabideau CM, Rowden SJL, Zhang JZ, Howe CJ. (2019) The development of biophotovoltaic systems for power generation and biological analysis. ChemElectroChem, in press.

Dorrell RG, Nisbet RER, Barbrook AC, Rowden SJL, Howe CJ (2019) Integrated genomic and transcriptomic analysis of the peridinin dinoflagellate *Amphidinium carterae* plastid. Protist 170:358-373.

Invited Presentations

University of Stellenbosch, August 2018 (Howe)
University of Muenster, December 2018 (Howe)
OpenPlant Forum, Cambridge July 2019 (Rowden)

Workpackage C: New mechanisms for Regulation of Gene Expression

Relationship to other projects/themes

This project relates to the following projects:

- Riboswitches in new chassis (C3) and Riboregulator circuits (C4) (Alison Smith). We are both interested in methods of transgene delivery into the green alga *Chlamydomonas reinhardtii* and in technology to control gene expression. We both participate in an international project for the construction of a Chlamydomonas MoClo kit of Golden Gate domesticated DNA parts, which will foster the development of synthetic biology in algae.
- Cyanobacteria circuits (B3) (James Locke). We share an interest in quantitative methods to measure gene circuit output.
- This OpenPlant project also benefits from present and past projects in the RNA silencing lab, which provide tools, mutant strains and methods.

Investigators

David Baulcombe (3 days); Alison Smith (Cambridge; 6 days)

Staff Employed

Francisco Navarro (PDRA; Baulcombe lab). Started May 2015 – Ended February 2018
Gonzalo I Mendoza-Ochoa (PDRA; Smith lab). Started October 2017 – Ended May 2019

Partners

An OpenPlant Fund grant has established new collaborations between OpenPlant PDRA Francisco Navarro and John Innes Centre bioinformatics specialist, Marielle Vigouroux, to develop a codon optimisation tool for Chlamydomonas.
Tim O'Leary, Department of Engineering, University of Cambridge

An OpenPlant Fund grant has established new collaborations between OpenPlant PDRA Gonzalo Mendoza, PhD student Aleix Gorchs and SRA Payam Mehrshahi (Cambridge), and OpenPlant PDRA Oleg Raitskin and PDRA Quentin Dudley (Earlham Institute, Norwich), to develop a method based on CRISPR/Cpf1/ssDNA for precise targeted transgene integration into the nuclear genome. The method will be optimized in Chlamydomonas and then tested in protoplasts of land plants.

Aims (C1-2)

1) To develop tools of gene expression control using the endogenous RNA silencing machinery of the green alga *Chlamydomonas reinhardtii*.
2) To design and construct synthetic gene circuits of increasing complexity using miRNAs.
3) To evaluate the mechanism of sRNA-dependent gene silencing in *Chlamydomonas* by quantitative methods.

Aims (C3)

1) Identify riboswitches from diverse organisms that have already been characterised and shown to regulate transgene expression in their native hosts.

2) Follow synthetic biology approaches to generate new expression platforms based these riboswitches, which will allow metabolite-inducible expression of transgenes.
3) Test the responsiveness of the novel riboswitches for the control of transgene expression in different photosynthetic eukaryotic organisms (including microalgae and plants).

Milestones

C1: RNA silencing modules for regulation of genes in a land plant and an alga.
Deliverable: Identification of endogenous siRNA and miRNA loci from a land plant and an alga that could serve as the backbone for construction of RNA silencing modules (month 6, Baulcombe).
Deliverable: Assembly of test modules incorporating the backbones and demonstration that they can be used to silence gene expression in a land plant and an alga (month 18, Baulcombe). Deliverable: Analysis of variant test modules in different tissue/growth states to characterize effective silencing systems and targeting rules (month 24, Baulcombe).

C2: Validation of RNA silencing modules for regulation of genes in a land plant and an alga.
Deliverable: Testing of RNA silencing modules for validation of artificial silencing systems with feedback based regulation (month 30, Baulcombe)

C3: Standardised riboswitch parts for transgene regulation in different chassis.
Deliverable: Set up a high-throughput workflow to analyse riboswitches for control transgene expression in algae (month 36, Smith (UCam)).

Deliverable: Generate a spectrum of streamlined and highly characterized riboswitches for controlled gene expression in algae (month 42, Smith (UCam)).
Deliverable: Public release of DNA parts encoding riboswitches for use in land plants and algae (month 48, Smith (UCam), Osbourn).

C4: Riboregulators for plastid transgene regulation.
Deliverable: Public release of DNA parts encoding TPR/PPR proteins characterised as components for regulation of plastid gene expression (month 60, Smith (UCam)).

Progress to date

C1-C2: Covered in previous report.

C3: PDRA Gonzalo Mendoza-Ochoa was recruited to the Smith lab at the University of Cambridge to work on milestone C3 until his departure in May 2019 to take up a Broodbank Fellowship in the Dept of Plant Sciences.

Previous work in the lab identified in Chlamydomonas thiamine-responsive RNA elements (thiamine riboswitches). With the knowledge gained, these splicing-dependent riboswitches were engineered and refined for precise control of transgene expression. Our aim here was to export these tools to other organisms (e.g. land plants). Based on preliminary results in

Saccharomyces cerevisiae, we predict that the high GC content of Chlamydomonas DNA may prevent riboswitches from Chlamydomonas working on other organisms. Because the riboswitch mechanism involves extensive and specific secondary structure, this cannot be overcome by modification of the riboswitch sequence. This is consistent with data of PhD student Marcel Llavero, which suggested that Chlamydomonas riboswitches were not directly functional in the diatom alga *Phaeodactylum tricornutum*, and indeed did not splice properly even without the ligand. This might mean that a riboswitch could require extensive modifications and optimisations before it can be used optimally in another host organism. Currently, we are focusing our efforts on optimizing and expanding our riboswitch tools in Chlamydomonas.

To be able to build and analyse novel riboswitches in *Chlamydomonas* in a high-throughput manner, we used the recently published *Chlamydomonas* Golden-Gate MoClo kit (Crozet et al 2018) to construct a series of riboswitch-regulated fluorescent reporter genes. This has allowed us to rapidly and directly quantify changes in expression levels upon activation of the riboswitch by thiamine supplementation. More generally, use of fluorescent proteins, as C-terminal tags of transgenes or as reporters of promotor activity, was only possible until recently and is now a widely use strategy in our lab for both *Chlamydomonas* and *Pheaodactylum* alga models.

Payam Mehrshahi has demonstrated the utility of the thiamine riboswitch in *Chlamydomonas* for metabolic engineering, by placing the casbene synthase enzyme under regulatory control of the riboswitch, which allowed tuneable production of casbene diterpenoid dependent on exogenous thiamine concentrations. The system was successful for either the nuclear or chloroplast encoded transgenic enzymes. Dr Mehrshahi, SRA Dr Matthew Davey and PhD student Nhan-An Tran are now attempting to scale up growth of the Chlamydomonas strain producting this high-value terpenoid in the Algal Innovation Centre under natural conditions.

C4: Covered in previous report.

Evidence of the quality of the research

Publications

Chung, B.Y.-W., Valli, A., Deery, M.J., Navarro, F.J., Brown, K., Hnatova, S., Howard, J., Molnar, A., and Baulcombe, D.C. (**2019**). Distinct roles of Argonaute in the green alga Chlamydomonas reveal evolutionary conserved mode of miRNA-mediated gene expression. Sci. Rep. 9: 1–12. doi: 10.1038/s41598-019-47415-x

Navarro FJ and Baulcombe D. (**2019**) miRNA-mediated regulation of synthetic gene circuits in the green alga Chlamydomonas reinhardtii. ACS Synth Biol. 8(2):358-370. doi: 10.1021/acssynbio.8b00393.

Other evidence of impact

Conferences

Gorchs Rovira A, Mehrshahi P and Smith AG. *Expanding the Chloroplast Toolbox in Chlamydomonas reinhardtii using TPR/PPR proteins*, Talk at GRS and poster at GRC on Chloroplast Biotechnology in Ventura CA in January 2019.

Mendoza-Ochoa GI, Mehrshahi P and Smith AG. *Thiamine riboswitches for controlling transgene expression in the alga Chlamydomonas reinhardtii.* Aptamers in Bordeaux 2019. Bordeaux, 28-29 Jun 2019.

Workpackage D: Genome Engineering

Relationship to other projects/themes

The molecular tools for genome engineering and large-scale gene assembly technologies developed in Workpackage D will be of direct benefit to all the trait-engineering work-package (F, G, H, I & J). Specifically, vectors for chloroplast manipulation and methods to achieve homoplasty are being developed for use in Workpackage F and molecular tools for targeted genome editing are being developed for use in Workpackages G, H and I.

Increased accessibility to engineering the *M. polymorpha* chloroplast will have direct implications on Workpackages: F4, F5 and C4, as they all involve chloroplast engineering.

The chloroplast-optimised fluorescent reporter library will be of direct use to several work packages, specifically F1-5, C3 and C4. Additional reporters will increase our ability to probe transcription/translation in plants. Additionally, standardised DNA assembly technologies from Workpackage B have been applied to the technologies developed in Workpackage D.

Investigators

Nicola Patron (10 days); Jim Haseloff (5 days); Jim Ajioka (4 days); Giles Oldroyd (1 day); James Locke (2 days); Christopher Howe (0.5 days); Alison Smith (JIC; 0.5 day); Alison Smith (UCam; 2 days); Sebastian Schornack (8 days); Julian Hibberd (1 day)

Staff Employed

Oleg Raitskin (PDRA; Patron lab). Start Jan 2015. Re-employed at EI Sep 2016
Douglas Griffith (PDRA, Locke lab). Start Jul 2015, end Nov 2016
David Willey (PDRA, Ajioka lab). Start Sep 2015, end Mar 2016
Orr Yarkoni (PDRA, Ajioka lab). Start May 2016, end date Feb 2018
Philip Carella (PDRA, Schornack lab). Start Sep 2016
Stephen Rowden (PDRA, Howe lab). Started Sep 2017
Gonzalo I Mendoza-Ochoa (PDRA; Smith lab). Started October 2017 – Ended May 2019

Partners

Several collaborations within OpenPlant have been established to accelerate the application of genome engineering technologies from Workpackage D to other Workpackages. Specifically, engineering the Marchantia genome in the Haseloff lab, UCam (Workpackage A) and engineering the potato genome in the Smith Lab, JIC (Workpackage G). The latter has resulted in a publication (Tuncel et al., 2019, Plant Biotechnology Journal).

Additionally, several collaborations outside of OpenPlant have been established:

A collaboration to establish a toolkit for Chlamydomonas between the Smith Lab (UCam) and laboratories in Paris, Copenhagen, and Bielefeld and Kaiserslautern resulted in a MoClo kit for the nuclear genome (Crozet et al., 2018, ACS Synth Biol).

A collaboration for extensive engineering of the Chlamydomonas chloroplast genome has been established between Smith (UCam) and Saul Purton (UCL). They have been awarded an BBSRC grant (BB/R01860X/1; Oct 2018 - Sept 2021).

A collaboration between Patron (EI), Siobhan Brady and David Segal (UC Davis) was established to engineer complex traits using targeted, multiplexed genetic and epigenetic mutagenesis. They were awarded a BBSRC/NSF/USDA grant (BB/S020853/1; July 2019-July 2021).

A collaboration between Patron (EI) and O'Connor (JIC) was awarded a BBSRC grant (BB/P010490/1; Oct 2017 – Sept 2020) to apply engineering tools and processes developed in Workpackage D for improving plants as bioproduction platform for proteins and small molecules. This is an Industrial partnership with Leaf Expression Systems. Related to this, Patron (EI) established a collaboration with the Geu-Flores Lab (University of Copenhagen) to edit genes in the plant alkaloid biosynthesis pathway to improve them as a production chassis for medicinal alkaloids.

A collaboration between Harwood (JIC) and Patron (EI) to provide targeted gene knockouts in crops using RNA-guided Cas9 nuclease to the UK research community was awarded funds from the BBSRC Bioinformatics and Biological Resources 2015 call (BB/N019466/1; Sep 2016 – August 2019).

Nathalia Volpi (Mazzo lab, University of Campinas, Brazil) obtained a FAPESP fellowship to conduct genome engineering experiments in the Patron Lab (EI) to elucidate carbon flux between lignin and chlorogenic acid. This fellowship was undertaken in 2016-2017. Following maternity leave, the fellow is now conducting the final analysis of these plants.

Milestones

D1: Construct and characterise yeast artificial chromosome vectors as plant shuttle systems.
Deliverable: Public release* of YAC or BAC based vectors for plant genome engineering (month 36, Ajioka, Patron).

D2: Plastid genome vectors for chloroplast transformation.
Deliverable: Public release* of vectors for efficient plastid transformation (month 36, Haseloff, Ajioka, Smith (UCam)).

D3: System for facilitating homoplasty after chloroplast transformation.
Deliverable: Public release* of tools and vectors for reverse host-restriction in plastids (month 60, Haseloff).

D4: Establish vectors for ds-break mediated gene deletion, mutation and addition.
Deliverable: Public release* of CRISPR/Cas9 or TALEN based tools to delete, mutate and deliver exogenous DNA to specific genomic loci in several model and crop plant species. (month 60, Schornack, Patron, Jones, Oldroyd, Hibberd, Haseloff).

Progress to date

D1-D3: Covered in previous report.

D4: Establish vectors for ds-break mediated gene deletion, mutation and addition.

Oleg Raitskin (Patron lab) has demonstrated RNA-guided Cas9-mediated targeted mutagenesis and gene deletion in several plant species (Nicotiana, Arabidopsis). This has also been established in the Schornack lab, where an internal access website for genome editing at UCam has been set up. Susana Sauret-Gueto has lead work in the Haseloff Lab to

design and build gRNA accepting Loop vectors (from Workpackage B), that can be directly used to generate mutants when transforming Cas9 Marchantia spores. The aim is to use this vector to accept a library of synthetic gRNAs for efficient targeted mutagenesis of Marchantia TF genes.

Oleg Raitskin (Patron lab) together with the Earlham DNA Foundry, has developed automated construct assembly and delivery to protoplasts followed by sequencing to enable rapid quantitative assessments of the efficiency and specificity of the constructs. A suite of variants of Cas proteins from different bacterial species was systematically compared resulting in an expanded standardized toolkit for genome engineering in plants (Raitskin et al, 2019, PloSOne). Methods for protoplast delivery developed in this study were also applied to potato (**Workpackage G**) in collaboration with Aytug Tuncel in the Smith lab (JIC). This resulted in a publication (Tuncel et al., 2019, Plant Biotechnology Journal).

Oleg Raitskin (Patron lab) has also produced transgenic plants (*Nicotiana tabacum, Nicotiana benthamiana* and *Arabidopsis*) with disrupted selection cassettes designed to enable efficient recovery of targeted insertion events through targeted delivery and repair. Simultaneous delivery of nucleases and the repair template to protoplasts has demonstrated the recovery of callus with targeted insertions on selective media. However, efficient regeneration of shoots from protoplast-derived callus is proving challenging.

Evidence of the quality of the research

Publications:

Dudley Q, Raitskin O, Patron NJ (*in press*) **Cas9-mediated targeted mutagenesis in plants** Methods in Molecular Biology

Tuncel A, Corbin KR, Ahn-Jarvis J, Harris S, Hawkins E, Smedley MA, Harwood W, Warren FJ, Patron NJ, Smith AM (2019) **Cas9-mediated mutagenesis of potato starch branching enzymes generates a range of tuber starch phenotypes**. *Plant Biotechnol J.* doi: 10.1111/pbi.13137. [Epub ahead of print] - ***Collaborative with Workpackage G***

Raitskin O, Schudoma C, West A, Patron NJ. (2019) **Comparison of efficiency and specificity of CRISPR-associated (Cas) nucleases in plants: An expanded toolkit for precision genome engineering.** *PLoS One.*14(2):e0211598. doi: 10.1371/journal.pone.0211598.

Other evidence of impact:

Invited talk (Patron) ALife, Newcastle, July 2019

Invited talk (Patron) Designer Biology, Newcastle, July 2019

Invited talk (Patron) Centre for research in agricultural Genomics, Barcelona, July 2019

Invited talk (Smith UCam) Synthetic biology for metabolic engineering in algae. NIPGR New Delhi, India, July 2019

Invited talk (Smith UCam) Synthetic biology for metabolic engineering in algae. IISER Pune, India, July 2019

Invited talk (Smith UCam) Exploiting microalgae for biotechnology. ICT-CEB, Mumbai India, July 2019

Invited talk (Smith, UCam) Riboswitches - plug-and-play devices for synthetic biology approaches to metabolic engineering. Universität Münster, June 2019

Thinking outside the flask – how to do something useful with algal metabolism. EPFL Switzerland, February 2019

Participation in an advisory committee (Patron) Expert member of the plant synthetic biology working group for the European Food Standards Agency (EFSA) (2019)

Participation in an advisory committee (Patron) Engagement with the Parliamentary Scientific Committee: implications of the 2018 European Court of Justice ruling on Genome Editing (2019) February 2019

Participation in an advisory committee (Patron) Engagement with the Parliamentary Scientific Committee: implications of the 2018 European Court of Justice ruling on Genome Editing (2019) February 2019

Participation in a workshop (Patron) Molecular Training for African Scientists, Cambridge February 2019

Invited talk and session chair (Smith, UCam) GRC Chloroplast Biotechnology conference, January 2019

Invited talk (Smith, UCam) Riboswitches - plug-and-play devices for synthetic biology approaches to metabolic engineering. Warwick Insitute for Synthetic Biology, December 2018

Invited talk (Patron) Revolutionizing Agriculture with Synthetic Biology, Banbury Centre, Cold Spring Harbor, December 2019

Invited talk (Patron) Plant Synthetic Biology, Bioengineering, and Biotechnology, Clearwater, Florida, December 2019

Invited talk (Patron) Carl Wrose Centre, University of Illinois, December 2018

Invited talk (Smith, UCam) Thinking outside the flask – doing something useful with algal metabolism. University of Leeds, November 2018

Invited talk (Smith, UCam) TPP riboswitches in algae – characterization, evolution & biotechnological potential. ENS, Paris, November 2018

Workpackage E: Digital Tools

Relationship to other projects/themes

Digital Tools provides underpinning technologies for modelling, DNA assembly and distribution of part data. This supports Work Packages A, B, D and others producing vectors, tools and parts.

Investigators

James Locke (1 days); Jim Haseloff (5 days); Jim Ajioka (0.5 days); Nicola Patron (0.5 days); Giles Oldroyd (0.5 days)

Staff Employed

Bruno Martins (PDRA, Locke lab). Started January 2017

Partners

Mihails Delmans; Bernardo Pollak

Aims

Software tools play an increasingly important role in Synthetic Biology experiments, as we automate experiments, and the systems we construct increase in scale. In order to accurately predict the behaviour of biological systems, which are governed by multiscale parallel and feedback regulated genetic, physical and chemical interactions we need computational models. This workpackage aims to provide software to automate DNA assembly and the quantification of gene expression in plant in addition to providing models for gene expression and cell growth.

Milestones

E1: Software for automated DNA assembly.
Deliverable: Implementation of software for a DNA assembly pipeline, in collaboration with Nathan Hillson, JBEI (month 12, Ajioka, Patron, Haseloff, Oldroyd).

E2: Software for automated quantification of gene expression in planta.
Deliverable: Public release* of open source software routines for automated processing of gene expression data in microbes and plants (month 24, Haseloff, Locke).

E3: Software models for gene circuits.
Deliverable: Web-based access to developments in modelling of gene circuits in the OpenPlant community, and web access to parameters of DNA parts via JBEI-ICE API (month 36, Haseloff, Locke).

E4: Software models for cell growth.
Deliverable: Public release* of open source software for multi-scale modelling of cellular growth in microbes and plants (month 60, Haseloff, Locke).

Progress to date

E1-6: Mihails Delmans (Haseloff Lab) has developed imaging and image-processing methods, which allow simultaneous tracking of tissue kinetics and dynamics of gene expression. An image-registration-based pipeline was extended to enable measurement of tissue expansion of Marchantia gemmae with sub-cellular resolution using the signal of a membrane-localised fluorescent protein. The same technique was applied to track the positions of the apical notches, which enabled normalisation and averaging of the spatial

patterns of tissue expansion and fluorescent protein gene expression. The algorithm allowed one to account for the natural variation in gemma size and enable the study of correlations between signals obtained from different Marchantia lines.

Monica Dayao, in collaboration with the Haseloff lab employed deep learning methods to create a reliable binary segmentation masks for labelling pixels in microscopy images as cell, or not cell. She developed methods to generate synthetic data to address the training data bottleneck for *M. polymorpha* biological datasets, and extended the binary segmentation to instance segmentation, where individual cells could be identified and classified.

Mihails Delmans (Haseloff lab) continued to maintain and extend the MarpoDB database.

The last year 2018-2019 has seen the publication of output from around 50 hardware and software projects (https://www.biomaker.org/projectindex), using the Biomaker platform at: https://www.hackster.io/biomaker/

Evidence of impact

Publications:

Boehm CR, Grant PK, Haseloff J. (**2018**). Programmed hierarchical patterning of bacterial populations. Nat Commun. 9(1):776. doi: 10.1038/s41467-018-03069-3.

Delmans M, Haseloff J, (**2018**). μCube: A Framework for 3D Printable Optomechanics. Journal of Open Hardware, 2. 10.5334/joh.8.

Kan A, Del Valle I, Rudge T, Federici F, Haseloff J. (**2018**). Intercellular adhesion promotes clonal mixing in growing bacterial populations. J R Soc Interface. 15(146). pii: 20180406. doi: 10.1098/rsif.2018.0406.

Martins BMC, Tooke AK, Thomas P, Locke JCW, (**2018**). Cell size control driven by the circadian clock and environment in cyanobacteria. Proc Natl Acad Sci U S A. 115(48):E11415-E11424. doi: 10.1073/pnas.1811309115.

Other evidence of impact:

Public access to MarpoDB website at http://marpodb.io

Public access to repository of open source software archives and information:

https://github.com/HaseloffLab

Public access to projects documented at: https://www.hackster.io/biomaker/

Many projects have been accessed thousands of times.

Workpackage F: Modules for engineering photosynthesis in leaf metabolism

Relationship to other projects/themes

Workpackage F aims to employ standardised DNA parts for the assembly of a collection of tools useful for engineering photosynthesis in plants. The will be strong interactions with the standards being established in Workpackages B and D, along with application of the parts in Workpackages A, G, H and J.

Investigators

Julian Hibberd (6 days); Alex Webb (5 days); Jim Haseloff (4 days); Alison Smith (JIC; 1 day)

Staff Employed

Ivan Reyna-Llorens (PDRA; Hibberd lab) Started Oct 2015.
Lukas Mueller (PDRA; Webb lab) Mar 2017- Feb 2019.
Eftychios Frangedakis (PDRA; Haseloff lab) Started April 2017.

Partners

Jim Ajioka; Nicola Patron; Christian Boehm

Aims

Plant leaves are biofactories that can accumulate valuable products in a number of discrete compartments both within and between cells. Furthermore, they also fine tune synthetic pathways in response to environmental signals. While significant progress has been made in defining cell specific gene expression in roots, this has not been achieved in leaves. This is a bottleneck in engineering this easily harvested organ, and there is no central repository of genetic modules to facilitate this. We aim to provide a library of elements that can be used to drive expression of both nuclear and plastid encoded genes in specific compartments of specific cells of leaves, and in addition to control that expression over the day-night cycle. These modules will be registered and made available in the OpenPlant repository.

Milestones

F1: Protein scaffolds for cell specific and targeted intracellular expression in leaves.
Deliverable: Artificial protein scaffolds from bacterial systems that can be assembled in planta. Public release* of DNA parts for scaffolds and cognate ligands (month 24, Hibberd).

F2: DNA motifs that generate cell specific expression in leaves.
Deliverable: Leaf specific promoter motifs will be identified by sequencing of RNAs from isolated cell types and functional testing, and released publically* (month 36, Hibberd).

F3: Transcription factors and target cis-elements for engineering co-ordinated expression of synthetic pathways in leaves.
Deliverable: Leaf specific transcription factors and characterised cis elements will be released publically* (month 36, Hibberd).

F4: Inducible and cell-specific expression of genes in the chloroplast genome
Deliverable: Plastid targeted systems for regulation of plastid gene expression will be characterised and released publically* (month 60, Webb, Haseloff).

F5: Circadian control of synthetic promoters and gene control in chloroplasts
Deliverable: Public release* of synthetic promoters for expression at defined phases in the day-night cycle in plants (month 60, Webb, Smith/JIC).

Progress to date

F1-F3: Covered in previous report. New publications are listed below

F4-F5: In addition to material covered in previous reports, Dr. Frangedakis (Haseloff lab) has constructed a series of Loop assembly vectors suitable for chloroplast transformation. These enable rapid construction of new genes that are suitable for delivery to the Marchantia chloroplast genome. The vectors are paired with a library of parts including target regions for homologous recombination, plastid promoters, terminators, selectable markers and marker genes. This set of parts has been tested using the optimised plastid transformation protocol established in the previous year.

Evidence of the quality of the research

Publications

Borba AR, Serra TS, Górska A, Gouveia P, Cordeiro AM, Reyna-Llorens I, Kneřová J, Barros PM, Abreu IA, Oliveira MM, Hibberd JM, Saibo NJM (**2018**). Synergistic binding of bHLH transcription factors to the promoter of the maize NADP-ME gene used in C4 photosynthesis is based on an ancient code found in the ancestral C3 state. Mol Biol Evol. 35(7):1690-1705. doi: 10.1093/molbev/msy060.

Burgess, S.J. Reyna-Llorens, I., Stevenson, S.R., Singh, P., Jaeger, K., Hibberd, J.M. (**2019**) Genome-wide transcription factor binding in leaves from C3 and C4 grasses. The Plant Cell doi.org/10.1105/tpc.19.00078

Workpackage G: Carbohydrate Engineering

Relationship to other projects/themes

This workpackage will use DNA assembly technologies and genome editing technologies developed in workpackages B and D. Nicola Patron is co-supervising the potato project described below.

Investigators

Paul Dupree (12 days); Rob Field (8 days); Alison Smith (JIC; 7 days); Nicola Patron (1 day)

Staff Employed

Aytug Tuncel (PDRA; Smith lab at JIC) Started Jan 2015, completed April 2018
Henry Temple (PDRA; Dupree lab) Started Feb 2017, completed March 2019
Louis Wilson (OpenPlant DTP student in Dupree lab) Started Apr 2017

Partners

Alison Smith obtained Norwich Research Park Innovation funds to establish a transformation method for potatoes in the JIC BRACT transformation group to support a CRISPR/Cas9-mediated carbohydrate engineering project. A collaboration with the Quadram Institute, Norwich, is providing access to state-of-the-art starch analytical techniques.

Research into the exploitation of algal carbohydrate-active enzymes, identified through OpenPlant, is supported by a £3.4M Innovate UK grant on 'Glycoenzymes for bio industries' - awarded jointly to University of Manchester, Newcastle University, Institute of Food Research (Norwich) and the John Innes Centre, in collaboration with industrial partners Ludger, Biocatalysts and Prozomix.

Paul Dupree is an Investigator in the Leverhulme Natural Material Innovation Centre, a £2M Leverhulme Trust project in the University of Cambridge, to improve materials from plants, such as timber, for building construction. This provides additional support to study the properties of plants engineered in OpenPlant, and assistance in the glucomannan biosynthesis studies. Jan Lyczakowski a Cambridge BBSRC DTP funded student in the PD lab who is affiliated to the OpenPlant programme, completed his PhD.

Aims

Plants provide unrivalled opportunities for provision of sugars and polysaccharides for biorefining, biofuels, animal feed, food and other industrial uses. The main goal of this workpackage is to improve the quality and increase the yield of target polymers, and to alter their structure for higher value applications. The targets will be plant cell wall polymers that important to these applications: xylan, mannan, and novel digestible glucans.

The objectives will be achieved by building a registry of polysaccharide synthesis pathway genes and transcription factors that can be co-ordinately expressed using tested promoters from this and other workpackages.

Current milestones

G1: A resource of inducible expression systems in fibre cells.
Deliverable: Public release* of fibre cell specific promoters (month 54, Dupree).

G2: A resource of genes for engineering ectopic mannan synthesis.
Deliverable: Public release* of glycosyltransferase gene tools for ectopic synthesis of mannans (month 60, Dupree).

G3: A resource of genes for engineering xylan synthesis.
Deliverable: Public release* of glycosyltransferase genes that direct modification of xylans in planta (month 60, Dupree).

G4: A tool-kit of algal glucan-active enzymes.
Deliverable: Public release* of carbohydrate-active enzymes mined from red algae genomes (month 24, Field).

G5: Cytosol-targeted expression of glucan-active enzymes.
Deliverable: Characterisation of new enzymes for glucan synthesis and modification in plants (month 36, Smith/JIC, Field).

G6: Engineered plants producing cytosolic glucans.
Deliverable: Transgenic plant lines as models for biosynthesis of novel glycans (month 36, Smith/JIC, Field).

Progress to date

Milestones **G1-3** started in year 3. In February 2017 PDRA Henry Temple joined the project. An OpenPlant DTP student Louis Wilson joined the project from May 2017.

Engineering polysaccharide synthesis depends greatly on using the right tools for the desired specific goals. After cellulose, the hemicelluloses xylan and mannan are the most abundant polysaccharides on the planet. This project aims to modify the synthesis of these cell wall components by modifying their synthesis in the Golgi apparatus. Using specific promoters driving effective and specific glycosyltransferase (GT) activities is the cornerstone to achieve it, therefore the Dupree group has tested different candidates of promoters and GT activities which have resulted in successful examples for re-engineering of cell wall polysaccharide synthesis.

Important differences exist among hemicellulose molecules of plants of different taxa. One example of this is the difference between conifer and eudicot xylan. While eudicot xylan molecules are greatly acetylated in their backbone, conifer xylan backbone does not contain acetylation. On the other hand, conifer xylan molecules are substituted extensively by 3-*O*-linked arabinose (Ara) groups, implying previously uncharacterised conifer Xylan ArabinosylTransferase (XAT) enzymes exist (Busse-Wicher et al., 2016). The presence of 2-*O*-linked glucuronic acids (GlcA) substitutions in the backbone is a common feature among dicots and conifers, but the patterning of them is different, and probably genetically controlled. Mortimer et al., 2010 showed that GlucUronic acid substitution of Xylan (GUX) enzymes are responsible for this activity in Arabidopsis. Again, the patterning is different in conifers and eudicots.

We have prepared constructs for expressing specific glycosyltransferases (GTs) activities using a panel of validated tissue-specific promoters that are specific to secondary cell wall synthesising cells. Constructs for plant transformation are made with OpenPlant Goldengate system. The constructs are used to test promoter and GT combinations for hemicellulose modification.

After *in silico* analysis using the tool for plant comparative genomics PLAZA (http://bioinformatics.psb.ugent.be/plaza/) we have identified potential conifer candidates for XATs which belong to the CaZy GT61 family (Anders et al., 2011). Phylogenetic analysis using several candidates of different species, show conifer putative XATs cluster in three

different groups. This analysis led us to choose candidates of each specific conifer group and we have cloned them into GoldenGate compatible vectors according to OpenPlant procedures. We have generated constructs for conifer putative XATs expression under specific promoters for secondary cell wall tissues. To test the activity of these putative XATs we have developed an *in vivo* strategy using specific *Arabidopsis* backgrounds (which possess specific modifications in xylan biosynthesis). We have successfully expressed conifer XATs in *Arabidopsis* plants and we have detected arabinosyltransferase, but also xylan xylosyltransferases (XyXTs) activities for members of all conifer GT61 groups.

A similar approach was used for conifer putative GUX enzymes. Phylogenetic analysis using several candidates of different species, show that conifer putative GUX cluster in two different groups. We have used the Golden gate cloning system with secondary cell wall-specific promoter parts, driving the expression of these conifer GUX enzymes. We have identified that both of GUX groups have glucuronic acid transfer activity onto xylan backbone, but one of them can transfer two consecutive glucuronic acids, as has been recently reported to occur in conifer xylan.

These findings show we have expanded the tools for engineering dicot secondary cell walls, generating novel xylan structures in Arabidopsis.

Furthermore, through interactions with others in the OpenPlant centre, we are using *N. benthamiana* heterologous expression with the pEAQ-HT vector system to express different GTs and then perform *in vitro* assays. Recently, we identified a *Picea glauca* GUX1 orthologue, which shows xylan glucuronidation activity *in vitro* after using *N. benthamiana* heterologous expression and *in vivo* in *Arabidopsis* plants (Lyczakowski et al., 2017). We are using this *in vitro* system to test the different Conifer XATs and XyXTs already shown to be active *in vivo*. This system has also allowed us to identify and characterise the specific activity of a Mannan α-Galactosyl Transferase1 (MAGT1) (Yu et al., 2018). We have consequently used this mannan branching GT activity to modify mannan in secondary cell walls of *Arabidopsis* plants.

G4: was met in the previous annual reports.

The Field group is currently exploring artificial, *in vitro* metabolic cycles, driving production of glucose-based oligosaccharides from cheap and readily available sucrose by using sucrose phosphorylase and glucan phosphorylases. We have now achieved this goal for oligosaccharides based on amylose (a-1,4-linked), cellulose (b-1,4-linked) and latterly b-1,3-linked glucan. In our search for the necessary but elusive b-1,3-glucan phosphorylase, we have identified two new families of carbohydrate-active enzymes from Euglena: a new family GH149 (Kuhaudomlarp et al., 2018) and GH161 (Kuhaudomlarp et al., 2019). These enzymes, which are only distantly related to the obvious GH94 b-1,4-glucan phosphorylases, are proving to be versatile tools for the generation of immune stimulatory b-1,3-glucans (up to dp ca 25) for evaluation in fish feed in a BBSRC/Newton Fund project with India and Bangladesh.

G5-6: PDRA Aytug Tuncel (Smith lab, contract finished April 2018) applied genome editing tools and technologies developed in the Patron lab (Workpackage D) to generate potatoes that putatively contain digestion-resistant starches with potential nutritional benefits (Tuncel et al 2019).

We designed genetic constructs to introduce targeted mutations into one or more copies of the genes encoding both isoforms of starch-branching enzyme (SBE). Aytug Tuncel also developed methods to transform and then regenerate protoplasts isolated from potato leaves. In many cases, this resulted in plants with mutations in the target genes without introducing a transgene into the potato genome.

Many of the starch granules from tubers from lines with mutations in only some copies of the SBE genes had apparently normal granules that arose from multiple hila (not seen in wild-type potatoes, in which granules arise from single hila). These lines also had very large numbers of extremely small granules, absent from wild-type tubers, and “knobbly” granules that appear to be fusions of multiple small granules. Cell-separation techniques using CDTA revealed that essentially every tuber cell contained a mixture of normal, multiple-hila, knobbly and tiny granules. Analysis of starch polymers using new UPLC-SEC analysis at the Quadram Institute revealed that, despite the abnormal granule phenotypes, starch from these lines had near-normal ratio of amylopectin to amylose. The starch granules from tubers from lines with in which all copies of the SBE target gene were mutated had fewer tiny and knobbly granules but each granule had deep fissures across the hilum. Analysis revealed these lacked the major amylopectin fraction. It consisted almost entirely of long, linear glucan polymers in the size range expected for amylose. We speculate that partial reduction of SBE activity profoundly affects granule initiation (leading to many tiny granules, multiple hila and knobbly granules) with only minor effects on amylopectin synthesis and the development of the granule matrix. Complete loss of SBE activity essentially eliminates normal amylopectin synthesis, leading to severe stresses within the granule matrix and the appearance of cracks and fissures. This work has been published (Tuncel et al 2019). The impact on the gut microbiome of starches produced in this study will shortly be investigated by the Warren Lab at the Quadram institute, using mice with humanised gut microbiomes.

We are evaluating the potential of cereal endosperm as platform for cytosolic expression of enzymes able to synthesise glucans. Unlike all other plant tissues, ADPglucose for starch synthesis is made in the cytosol rather than the plastid of endosperm cells, then imported into the amyloplast for starch synthesis. We investigated whether the cytosolic ADPglucose pool could support the synthesis of α1,4-glucans in the cytosol by engineering bread wheat to produce a glucan synthase from Agrobacterium (glgA) exclusively in the cytosol of cells in the developing endosperm (by employing a HMW glutenin promoter). This exercise was successful in that high activities of glgA and consequent diversion of the ADPglucose pool in the cytosol were achieved. However the main product accumulating in these grains was maltose, and not a glucan. Maltose levels were extremely high, with profound osmotic, metabolic and developmental consequences for the grain. The most likely explanation for this phenomenon is that the glucan product of glgA is degraded to maltose by a β-amylase located in the cytosol. Examination of wheat transcriptome databases revealed that only one of the several β-amylases encoded in the wheat genome is both putatively cytosolic and highly expressed during the relevant developmental window in the grain. The JIC-located exome-sequenced TILLING population for wheat contains mutations in all three homoeoalleles encoding this amylase, opening up the possibility of eliminating it in the transgenic line through crossing. This should give cytosolic accumulation of a linear α1,4-glucan, which could be modified through introduction of other glucan metabolising enzymes. The work was in part funded by BBSRC grant BB/K006517/1. It is in preparation for publication.

Evidence of the quality of the research

Publications

Wangpaiboon K, Padungros P, Nakapong S, Charoenwongpaiboon T, Rejzek M, Field RA, Pichyangkura R, (**2018**). An a-1,6-and a-1,3-linked glucan produced by Leuconostoc citreum ABK-1 alternansucrase with nanoparticle and film-forming properties. Sci Rep. 8(1):8340. doi: 10.1038/s41598-018-26721-w.

Kuhaudomlarp S, Pergolizzi G, Patron NJ, Henrissat B, Field RA (**2019**) Unraveling the subtleties of β-(1→3)-glucan phosphorylase specificity in the GH94, GH149 and GH161

glycoside hydrolase families. J Biol Chem. 2019 Apr 19;294(16):6483-6493. doi: 10.1074/jbc.RA119.007712.

Kuhaudomlarp S, Walpole S, Stevenson CEM, Nepogodiev SA, Lawson DM, Angulo J, Field RA (**2019**). Unravelling the Specificity of Laminaribiose Phosphorylase from Paenibacillus sp. YM-1 towards Donor Substrates Glucose/Mannose 1-Phosphate by Using X-ray Crystallography and Saturation Transfer Difference NMR Spectroscopy. Chembiochem. 20(2):181-192. doi: 10.1002/cbic.201800260.

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Tuncel A, Corbin KR, Ahn-Jarvis J, Harris S, Hawkins E, Smedley MA, Harwood W, Warren FJ, Patron NJ, Smith AM (**2019**) Cas9-mediated mutagenesis of potato starch branching enzymes generates a range of tuber starch phenotypes. Plant Biotechnol J. doi: 10.1111/pbi.13137. [Epub ahead of print]

Wagstaff BA , Rejzek M , Kuhaudomlarp S, Hill L, Mascia I, Nepogodiev SA, Field RA (**2019**) Discovery of an RmlC/D fusion protein in the microalga Prymnesium parvum and its implications for NDP-β-l-rhamnose biosynthesis in microalgae. J Biol Chem. 294(23):9172-9185. doi: 10.1074/jbc.RA118.006440.

Workpackage H: Tools for Engineering Plant Natural Products

Relationship to other projects/themes

Within Workpackage H, the HyperTrans transient plant expression, originally developed for expression of structural proteins (e.g. vaccines, antibodies; Workpackage J), has been developed as a highly effective platform for genomics-driven rapid prototyping of new metabolic pathways from plants and for combinatorial biosynthesis. This, in combination with customised algorithms for genome mining (WPH) and standards for DNA parts and assembly (WPB), has paved the way for the establishment of a powerful pipeline that will enable the chemical potential hidden in plant genomes to be translated into chemicals in sufficient quantities for structural determination by NMR and evaluation of bioactivity. The Global Garden workshop forum developed in WPL provides a simulating meeting place for consideration and discussion of the societal and commercial aspects relating to responsible stewardship and use of the world's plants and associated the regulatory frameworks to support Access and Benefit Sharing (Convention on Biological Diversity, the Nagoya Protocol.

Investigators

Cathie Martin (5 days); Anne Osbourn (8 days); Sarah O'Connor (4 days)*

*Sarah O'Connor left the project 30 June 2019 to move to the Max Planck Institute of Chemical Ecology, Jena

Staff Employed

Past:

Yang Zhang (PDRA; Martin lab at JIC). January 2015 – January 2016
Don Nguyen (PDRA; O'Maille lab at JIC). February 2015 – February 2016
Hans-Wilhelm Nützmann (PDRA; Osbourn lab at JIC). September 2014 – September 2017
Noam Chayut (PDRA; Martin lab at JIC). August 2016 – December 2017
Benjamin Lichman (PDRA; O'Connor lab at JIC). February 2016 – November 2018
Ingo Appelhagen (PDRA; Martin lab at JIC). January 2018 – February 2019

Current:

Michael Stephenson (PDRA; Osbourn lab at JIC). Started February 2015
Zhenhua Liu (PDRA; Osbourn lab at JIC). Started November 2017

Partners

Dr Marnex Medema, Dr Eric Schranz, University of Wageningen, the Netherlands
Unilever
Syngenta
Professor Beth Sattely, Stanford University
Norfolk Plant Sciences has been granted a patent in the USA on use of AtMYB12 to modulate metabolism.

Aims

Plants produce a rich and diverse array of natural products. These compounds have important ecological functions, providing protection against pests, diseases, ultraviolet-B damage and other environmental stresses. They are also exploited as pharmaceutical drugs, agrochemicals, within the food and drink industry, and for a wide variety of other industrial

biotechnology applications. Although plants are potentially a tremendous source of diverse and valuable natural products, identifying the pathways for the synthesis of these compounds is more complicated than in microbes because the genomes are larger and more complex. However, advances in sequencing technology coupled with the recent discovery that the genes for natural products pathways are in many cases organised in operon-like clusters within plant genomes; now makes it possible to access the genes and enzymes of specialised metabolism in plants far more readily. We aim to harness and exploit metabolic diversity using synthetic biology approaches.

Milestones:

H1: A database and resource of parts for enzyme building blocks for natural product synthesis.
Deliverable: Genome mining data and public release* of plant DNA parts for synthesis and modification of natural product synthesis (month 60, Osbourn, Martin, O'Connor).

H2: Optimised enzymes for terpene production.
Deliverable: Public release* of DNA parts encoding improved enzymes for terpene synthesis (month 36, O'Maille, Osbourn).

H3: Transcription factors for control of natural product production.
Deliverable: Public release* of DNA parts encoding transcription regulators for terpene and alkaloid synthesis (month 60, Osbourn, Martin).

H4: Synthetic metabolons for improved phenylpropanoid production
Deliverable: Characterisation of model synthetic metabolons for phenylpropanoid biosynthesis (month 60, Martin).

H5: Synthetic metabolic clusters for deployment into crop plants
Deliverable: Use of the synthetic metabolon toolkit for rapid assembly and testing in Marchantia and Arabidopsis (month 36, Osbourn).

Progress to date

H1: Using algorithms such as plantiSMASH, developed in collaboration with Dr Marnix Medema (University of Wageningen) and other in-house genome mining methods we are able to rapidly identify new candidate genes and pathways for the biosynthesis of different types of plant natural products. The transient plant expression platform that we have developed has greatly accelerated functional analysis of new enzymes and pathways, and through scale-up using our custom-built vacuum agro-infiltration system we are able to generate milligram to gram-scale quantities of compounds for structural analysis and for evaluation of biological activity (see publications). Our focus has been on triterpenes, one of the largest and most structurally diverse classes of plant natural products with important medicine, agricultural and other industrial applications. Characterized genes and enzymes for the biosynthesis and diversification of different triterpene scaffolds have been assembled into a triterpene toolkit. Development of a database and resource of parts for enzyme building blocks for triterpene synthesis is being prepared for public release. The triterpene toolkit will not only enable reconstitution of pathways for naturally occurring molecules but will also allow generation of suites of new-to-nature triterpenes by combinatorial biosynthesis. Zhenhua Liu (Osbourn group) has recently exploited available genome sequence resources to carry out a large-scale investigation of triterpene biosynthesis across the Brassicaceae (Liu et al., submitted). Oxidosqualene cyclases (OSCs) catalyse the first

committed step in triterpene biosynthesis. Systematic analysis of 13 sequenced Brassicaceae genomes was performed to identify all OSC genes. The genome neighbourhoods (GNs) around a total of 163 OSC genes were investigated to identify Pfam domains significantly enriched in these regions. All-vs-all comparisons of OSC neighbourhoods and phylogenomic analysis were used to investigate the relatedness and likely evolutionary history of the numerous candidate triterpene biosynthetic gene clusters (BGCs) observed. Functional analysis of three representative BGCs was carried out and their triterpene pathway products elucidated. Our results indicate that plant genomes are remarkably plastic, and that dynamic GNs generate new biosynthetic pathways in different Brassicaceae lineages by shuffling the genes encoding a core palette of triterpene diversifying enzymes, presumably in response to strong environmental selection pressure. These results illuminate a genomic basis for diversification of plant specialized metabolism through natural combinatorics of enzyme families, which can be mimicked using synthetic biology to engineer diverse bioactive molecules. This type of large-scale multi-genome analysis holds considerable potential for rapidly unlocking genes and enzymes for metabolic pathway engineering and diversification.

PDRA Zhenhua Liu (Osbourn group) has taken a further step to explore the genetic basis how plant BGCs are maintained and diversified at the within species level. His focus has been on the first characterized thalianol cluster in model plant, *Arabidopsis thaliana*. *A. thaliana* has been the first plant species to discover detailed whole-genome sequence variation in over 1000 natural accessions. Zhenhua tracked down the evolution of the thalianol cluster at both the between and within species level. His results revealed dynamic genetic drivers underlying the formation, maintenance and decay of the thalianol cluster. He detected past positive selection operating on a subset of sites, but purifying selection is most likely dominating the maintenance of this pathway at recent evolutionary time, presumably due to its important ecological functions. He also identified significant non-randomly associated SNP pairs in the thalianol cluster in comparison to non-clustered region. This provides the first genetic evidence supporting co-inheritance of metabolic gene clustering in plants. This work thus provides an example to understand the progression of birth, life and death of other diverse BGCs which are uniquely present in constrained plant species.

PDRA Michael Stephenson (Osbourn Group) has developed a systematic approach to mining the *Reaxys Natural Product Databases* (RNPD), allowing the surveying of natural oxidation diversity of triterpene scaffolds. He has applied this to the oleanane scaffold (beta-amyrin) revealing that every available position is oxidised in nature, and that there are large differences in the relative occurrence of specific oxidations with a pattern suggestive of a strong influence of substrate bias in the evolution of oleanane oxidases. Michael has developed Unix based tools to process the large raw datasets outputted from RNPD searches. He has used this to produces list of plant species that oxidase the oleanane scaffold at orphan positions (those for which oxidases are not currently known to act). He is using this data to focus searches for new enzymatic tools for oxidising this pharmaceutically important scaffold. The current target is a species producing an oleanane metabolite oxidised at two of the rarest orphan positions. Using *de novo* assembly of publicly available RNA-seq data, differential expression analysis, with self-organising tree analysis and hierarchal clustering methods, Michael has identified short-lists of candidate CYP450s similarly expressed to putative oxidosqualene cyclases (OSCs) in different tissues. Exploration of a root specific list has already revealed two CYP450s capable of oxidizing the oleanane scaffold at different positions. Michael has also applied this chemoinformatic approach to surveying the steroidal scaffold as part of a BBSRC Networks in Industrial Biotechnology and Bioenergy business interaction voucher-funded project with GlaxoSmithKline.

In, addition Michael has established the function of number of enzymes in other collaborative projects. Most notably in this reporting period, enzymes responsible for protolimonoid biosynthesis (Hodgson *et al.* PNAS), avenacin biosynthesis (Leveau *et al.* New Phytol), (Louveau *et al.* Plant Cell), (Orme *et al.* PNAS, submitted), and enzymes comprising a novel gene cluster in the Brassicaceae (Liu *et al*., submitted). Michael has also cloned and functionally screened 20 phylogenetically distinct OSCs from a large phylogenetic tree comprising several thousand sequences produced by a PhD student Charlotte Owen (Osbourn Group).

Furthermore, Michael has contributed to a book chapter on engineering tobacco for natural product production (Stephenson *et al*. Comprehensive Natural Products III, in press). He has also published a highly critical highlight article (Stephenson *et al.* Natural Product Reports). The highlight article presents the findings of an earlier collaborative project from a more chemical prospective then the original publication, and discusses the wider contextual significance of the novel triterpene stereochemistry discovered in this project. A stereochemistry that represents a potentially transformative discovery in triterpene biosynthesis, breaking a dogmatic dichotomy which has existed for over 60 years.

H2-5: Covered in previous report.

Evidence of the quality of the research

Publications

Stephenson MJ, Field RA, Osbourn A. (2019). The protosteryl and dammarenyl cation dichotomy in polycyclic triterpene biosynthesis revisited: has this 'rule' finally been broken? *Natural Product Reports* 36: 1044-1052.

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Liu Z, Suarez Duran HG, Harnvanichvech Y, Stephenson MJ, Schranz ME, Nelson D, Medema MH, Osbourn A. Drivers of metabolic diversification: how dynamic genomic neighborhoods generate new biosynthetic pathways in the Brassicaceae. Submitted.

Hodgson H, De La Pena R, Stephenson MJ, Thimmappa R, Vincent JL, Sattely E, Osbourn A. (2019) Identification of key enzymes responsible for protolimonoid biosynthesis in plants: Opening the door to azadirachtin production. *PNAS* 116:17096.

Leveau A, Reed J, Qiao X, Stephenson MJ, Mugford ST, Melton RE, Rant JC, Vickerstaff R, Langdon T, Osbourn A (2019). Towards take-all control: a C-21β oxidase required for acylation of triterpene defence compounds in oat. *New Phytol.* 221:1544.

Louveau T, Orme A, Pfalzgraf H, Stephenson M, Saalbach G, Hemmings AM, Leveau A, Rejzek M, Vickerstaff RJ, Langdon T, Field RA, Osbourn A (2018). Analysis of two new arabinosyltransferases belonging to the Carbohydrate-Active Enzyme (CAZY) glycosyl transferase family 1 provides insights into disease resistance and sugar donor specificity. *Plant Cell* 30: 3038.

Dangl J, Osbourn A, Orzaez D et al. (2018). What is the next frontier in plant engineering? *Cell* 174(3): 499.

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Hems ES, Wagstaff BA, Saalbach G, Field RA. (**2018**). CuAAC click chemistry for the enhanced detection of novel alkyne-based natural product toxins. Chem Commun (Camb). 54(86):12234-12237. doi: 10.1039/c8cc05113e. Erratum in: Chem Commun (Camb). 54(93):13161.

Huang A. C., Jiang T., Liu Y. X., Bai Y. C., Reed J., Qu B., Goossens A., Nützmann H. W., Bai Y., Osbourn A. (**2019**) A specialized metabolic network selectively modulates Arabidopsis root microbiota. Science 364(6440). pii: eaau6389. doi: 10.1126/science.aau6389.

Huang AC, Osbourn A. (**2019**) Plant terpenes that mediate below-ground interactions: prospects for bioengineering terpenoidsfor plant protection. Pest Manag Sci. 2019 Mar 18. doi: 10.1002/ps.5410. [Epub ahead of print]

Li Y, Wang H, Zhang Y, Martin C. (**2018**). Can the world's favorite fruit, tomato, provide an effective biosynthetic chassis for high-value metabolites? Plant Cell Rep. 37(10):1443-1450. doi: 10.1007/s00299-018-2283-8.

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Martin C (**2018**) A role for plant science in underpinning the objective of global nutritional security? Ann Bot. 122(4):541-553. doi: 10.1093/aob/mcy118.

Soubeyrand E, Johnson T, Latimer S, Block A, Kim J, Colquhoun TA, Butelli E, Martin C, Wilson MA, Basset G (**2018**). The Peroxidative Cleavage of Kaempferol Contributes to the Biosynthesis of the Benzenoid Moiety of Ubiquinone in Plants. Plant Cell. 30(12):2910-2921. doi: 10.1105/tpc.18.00688.

Stewart C, Patron N, Hanson A, Jez J, (**2018**). Plant metabolic engineering in the synthetic biology era: plant chassis selection. Plant Cell Rep. 37(10):1357-1358. doi: 10.1007/s00299-018-2342-1.

Wulff-Vester A, Wendell M, Matros A, Appelhagen I, Martin C, Hvoslef-Eide AK (**2018**) ANTHOcyanin production Platform Using Suspension Cultures in custom-made bioreactors. IN VITRO CELLULAR & DEVELOPMENTAL BIOLOGY-PLANT. 2018: 54, S77-S77

Xu JJ, Fang X, Li CY, Zhao Q, Martin C, Chen XY, Yang L. (**2018**) Characterization of Arabidopsis thaliana Hydroxyphenylpyruvate Reductases in the Tyrosine Conversion Pathway. Front Plant Sci. 9:1305. doi: 10.3389/fpls.2018.01305.

Other evidence of impact

PDRA Zhenhua Liu has given a selected talk at Terpnet 2019, Halle, Germany, August 2019.

Career development

This summer PDRA Michael Stephenson was awarded an Honorary Lectureship by the School of Chemistry, University of East Anglia, for his contribution to teaching over the past 4 years.

Workpackage I: N2 Fixation

Relationship to other projects/themes
Workpackage I feeds into Workpackage A, assisting in the establishment of Marchantia as a simple plant chassis for synthetic biology through the development and testing of methods and tools, and Workpackage B by producing parts that can be included in the parts collection. Workpackage I tests and uses genome editing tools produced in Workpackage D.

Investigators
Giles Oldroyd (5 days); Jim Haseloff (3 days); Sebastian Schornack (5 days);

Staff Employed
Pierre-Marc Delaux (PDRA; Oldroyd lab). September 2014 - August 2015
Philip Carella (PDRA, Schornack lab). Started September 2016
Chengwu Liu (PDRA; Oldroyd lab). Started February 2018

Partners
Gates-funded ENSA project (Engineering Nitrogen Symbiosis for Africa)
Three OpenPlant Fund grants have been funded for new collaborations between the Oldroyd group and groups in Cambridge, to explore the evolution of symbiosis signalling using Marchantia paleacea as a model, to develop modules for studying LysM receptor-like kinases and to develop novel cell reporters for high resolution imaging.

Aims
We have initiated an engineering strategy to transfer the recognition of rhizobial bacteria from legumes to cereals, as the first step towards engineering N-fixing cereal crops. This is a strategically important challenge and this Gates and BBSRC-funded programme represents one of the most ambitious engineering strategies in plant signalling. Marchantia provides a fantastic platform for testing synthetic biology approaches in engineering symbiosis signalling that is directly linked to a strategic programme in cereals.

Milestones
I1: Establishment of Marchantia as a model system for signalling in symbiosis.
Deliverable: Description of laboratory co-cultivation and marker techniques for symbiotic interactions between Marchantia spp. and Glomermycota fungi (month 12, Oldroyd, Haseloff, Schornack).

I2: Assembly of genetic components required for engineering Nod factor signalling.
Deliverable: A toolkit of transcription factors and signalling components for engineering synthetic responses to Nod factors (month 24, Patron, Oldroyd).

I3: Optimisation of gene circuits for synthetic Nod factor signalling in Marchantia.
Deliverable: Transgenic lines for optimising the function and activity of an ectopic Nod signalling pathway in (month 36, Oldroyd).

Progress to date

I1: Using the *M. paleacea* genome as a foundation for comparison of early divergent land plants, we have explored the extent to which the genetic components defined in legumes are necessary for symbioses in very diverse plant species. Previous work has demonstrated extensive gene loss with loss of symbiotic associations and consistently we found loss of the symbiosis signalling pathway components and loss of genetic components required for symbiont infection associated with loss of intracellular symbioses in many species of plants. However, very interestingly, in multiple cases of symbiont switches, i.e. loss of arbuscular mycorrhizal associations, but gain of a different symbiont, we always found conservation of these symbiotic genetic frameworks. This only occurred in species possessing intracellular

associations and was not conserved in species forming interceullar associations, for instance species forming ectomycorrhizal associations. This implies that the genetic frameworks that evolved in early land plants to support arbuscular mycorrhizal associations have been repeatedly utilised across the 450 million years of plant evolution to support the totality of intracellular symbioses. Consistent with these observations we have demonstrated that Sym pathway components of *M. paleacea* are fully conserved at the biochemical and functional level with their orthogous counterparts in legumes. This work has been deposited on bioarchive and is currently under review.
In parallel with this work we have also been attempting to establish Cas9 knockout mutation in *M. paleacea*. This has proven surprisingly challenging, but through a process of trial and error, we have finally managed to generate some mutants. These mutants, along with promoter reporter constructs are allowing us to validate that the symbiosis signalling pathway is invariantly conserved in early divergent plant species.

I2: From the research described above and research funded outside of OpenPlant, it is now very evident that there are few to no innovations in the Sym-pathway that evolved in legumes to facilitate the emergence of nodulation. This is consistent with the invariant conservation of the Sym pathway across the plant kingdom. This implies that the key innovations that allowed emergence of nodulation are outwith the Sym pathway. From multiple lines of evidence, we believe the key innovations that allowed emergence of nodulation in legumes were associated with the receptors that allow Nod factor recognition and the transcriptional regulators that modulate root growth to activate nodule organogenesis. There must also be genetic components that specifically allow rhizobial colonisation, but these have not been fully defined. *M. paleacea* is a very poor model for testing nodule organogenesis, since the species lacks roots, but is a good model for receptor engineering. Introduction of the legume receptors alond does not confer Nod factor recognition and versions of engineered receptors based on the receptors present in *M. paleacea* are now being tested.

I3: We are not at a stage for optimising Nod factor signalling. Rather we are attempting to engineer native receptors to allow Nod factor recognition.

Evidence of the quality of the research

Publications

Carella P, Evangelisti E, Schornack S (**2018**). Sticking to it: phytopathogen effector molecules may converge on evolutionarily conserved host targets in green plants. Curr Opin Plant Biol. 44:175-180. doi: 10.1016/j.pbi.2018.04.019.

Carella P, Gogleva A, Hoey DJ, Bridgen AJ, Stolze SC, Nakagami H, Schornack S. (**2019**) Conserved Biochemical Defenses Underpin Host Responses to Oomycete Infection in an Early-Divergent Land Plant Lineage. Current Biology 29(14): 2282-2294.e5 https://doi.org/10.1016/j.cub.2019.05.078

Radhakishnan G, Keller J, Rich M, Vernie T, Mbaginda D, Vigneron N, Cottret L, Clemente H, Libourel C, Cheema J, Linde A, Eklund M, Cheng S, Wong G, Lagercrantz U, Li F, Becard G, Oldroyd G, Delaux PM. (**2019**). The symbiosis signalling pathway is conserved in plant lineages forming intracellular symbioses. bioRxiv 804591 and under review

Workpackage J: Virus-based systems for bioproduction

Relationship to other projects/themes

Workpackage J provides a plant expression technology for use by others within the OpenPlant consortium and throughout the world. The technology is already well integrated in other workpackages, underlying the work of Workpackage H and also critical to research in Workpackage G. Work to extend the range of hosts for protein expression is carried out through interaction with Workpackage A.

Investigators

George Lomonossoff (5 days); Anne Osbourn (0.5 days)

Staff Employed

Eva Thuenemann (PDRA; Lomonossoff Lab). Started Nov 2014; Returned to work following maternity leave in August 2017 on a part-time basis (22.5 hours per week). End date 09 July 2018.

Partners

LeafSystems® International Limited

Aims

The CPMV-HT technology, and its associated pEAQ vectors (Sainsbury and Lomonossoff, 2008; Sainsbury et al., 2009), developed by George Lomonossoff (JIC) has established a unique position for the UK for rapid transient expression of proteins in plants through Agrobacterium-mediated infiltration of Nicotiana benthamiana leaves. CPMV-HT is a highly flexible system and will be developed for a range of applications in the field of plant synthetic biology.

Milestones

J1: A series of expression vectors with defined translational characteristics.
Deliverable: Distribution of new viral expression cassettes with fine-tuned levels of translation efficiency (month 24, Lomonossoff, Osbourn).

J2: Modification of the CPMV-HT system to permit expression in alternative hosts.
Deliverable: Distribution of new viral expression cassettes with extended host ranges (month 48, Lomonossoff).

J3: Methods for the delivery of expression vectors to a variety of hosts.
Deliverable: Distribution of new bacterial strains for the intracellular delivery of the viral vectors to a range of new plants hosts (month 60, Lomonossoff).

Progress to date

J1: A series of vectors have been developed in the Lomonossoff lab to enable fine-tuning of protein expression levels by making changes in the 5'- and 3'-UTRs of the CPMV-HT system (Meshcheriakova et Al., 2014, Plant Biotechnol J). Versions of the CPMV-HT system that are compatible with the OpenPlant plant common syntax (Pub 9) have been developed by the Patron and Osbourn groups.

More recently, a new synthetic version of the 5' UTR used in HT system has been developed and shown to be twice as effective as the original HT sequence. This work also revealed that the 3' UTR derived from CPMV RNA-2 is probably optimal and difficult to improve upon. Two vectors (pHRE and pHREAC) have been finalised and are ready for distribution, pending discussions with the involved stakeholders. It is hoped that the new synthetic version of the HT system can be distributed under an open MTA via OpenPlant. The pHREAC vector has proved to an effective way of expressing RNAs which, unlike RNA produced by pEAQ, have no residual ability to be replicated and thus cannot be packaged within CPMV particles. This

is important for the production of CPMV particles containing bespoke RNAs. Such particles have great potential as diagnostic controls and have attracted attention from commercial entities. They are also useful for structural analyses of RNA molecules by such techniques as SAXS and Raman spectroscopy (in collaboration with the University of Manchester, UK).

In collaboration with the Centre for Bioengineering at the Russian Academy of Sciences (CB-RAS), a new vector system (pEFF) has been developed a new which combines the high translational benefits of the CPMV-HT system with the replication ability of potato virus X (PVX). This system will be extremely useful in cases where virus spread throughout a host is desirable. It has also been shown recently that the presence of replicating RNA greatly facilitates the assembly of filamentous viruses. This will facilitate structural analysis of such particles and their use for the display of antigenic sequences.

J2: The CPMV-HT system has been used successfully in the BY2 cell pack system (developed by Fraunhofer Institute, Aachen, Germany) by the Lomonossoff group, is being used in Tomatoes by the Martin lab, and is being tested in Marchantia in collaboration with the Haseloff lab. The HyperTrans plant expression system has proved to be highly amenable for expression of plant natural product biosynthetic pathways, and has been integrated into the Osbourn lab's synthetic biology pipeline. A recent publication in the Journal of Visual Experimentation (Stephenson et al., 2018) provides a video introduction to the full pipeline and the accompanying methods. The Dupree lab are also utilising the hypertrans system to express different glycosyltransferases for use in in vitro assays. Research on the use of the hypertrans system for the expression of proteins in micro-algae is currently underway in collaboration with Sogang University, S. Korea

J3: Covered in previous report.

Evidence of the quality of the research

Publications

Berardi A, Baldelli Bombelli F, Thuenemann EC, Lomonossoff GP, (**2019**). Viral nanoparticles can elude protein barriers: exploiting rather than imitating nature. Nanoscale. 11(5):2306-2316. doi: 10.1039/c8nr09067j.

Hesketh EL, Tiede C, Adamson H, Adams TL, Byrne MJ, Meshcheriakova Y, Kruse I, McPherson MJ, Lomonossoff GP, Tomlinson DC, Ranson NA. (**2019**) Affimer reagents as tools in diagnosing plant virus diseases. Sci Rep. 2019 May 17;9(1):7524. doi: 10.1038/s41598-019-43945-6. PMID: 31101847

Matthew J. Byrne1, John F.C. Steele,, Emma L. Hesketh, Miriam Walden, Rebecca Thompson, George P. Lomonossoff* & Neil A Ranson1*. (**2019**) Combining transient expression and cryo-EM to obtain high resolution structures of luteovirid particles. Structure, https://doi.org/10.1016/j.str.2019.09.010

Peyret H, Brown J, Lomonossoff G (**2019**) Improving plant transient expression through the rational design of synthetic 5' and 3' untranslated regions. Plant Methods 15: 108 http://dx.doi.org/10.1186/s13007-019-0494-9

Workpackage K: OpenPlant Fund for interdisciplinary exchange

Relationship to other projects/themes
The OpenPlant Fund provides support for projects relevant to all work packages and fosters interdisciplinary exchange within and between the teams working on the different packages at each OpenPlant institution. The open hardware development and training component also especially supports OpenPlant pathways to international exchange.

Timing
Started September 2014 (Month 1)

Investigators
Jim Haseloff (4 days), Anne Osbourn (1 day)

Staff Employed
Jenny Molloy (Project Coordinator). Started February 2015 – Ended February 2018.
Colette Matthewman (Project Manager). Started October 2014 – Ended February 2019.
Alexandra Ting (Communications Officer for OpenPlant and SynBio SRI). Started January 2017 – Ended September 2019.
Dieuwertje van der Does (Project Manager). Started February 2019.

Partners
Cambridge Synthetic Biology Strategic Research Initiative (SynBioSRI)
Oliver Hadeler, Programme Manager of CamBridgeSens and the Sensor CDT, Department of Chemical Engineering and Biotechnology
Cambridge Consultants (Representative has contributed in-kind technical assistance to OpenPlant Fund teams and participated in judging panels)
Emre Ozer, Principal Research Engineer, ARM, Cambridge
Stefanie Reichelt, Head of Light Microscopy at Cancer Research UK, Cambridge
Alexandre Kabla, Department of Engineering, University of Cambridge
Dan MacLean, The Sainsbury Laboratory, Norwich
Microsoft Research (Representative has contributed in-kind technical assistance to OpenPlant Fund teams teams and participated in judging panel)
New England Biolabs (offered in-kind support to Biomaker Challenge teams)

Aims
The OpenPlant Fund was established to support seed projects on a competitive basis through the annual distribution of up to twenty £5000 grants following a lightweight application process and public pitching event. The aim of the fund is to promote the development of plant Synthetic Biology as an interdisciplinary field and to facilitate exchange between The University of Cambridge, the John Innes Centre and The Sainsbury Laboratory for the development of open technologies and responsible innovation in the context of Synthetic Biology.

Also, within in this work package we aim to promote open source hardware for science through supporting technical development and also the necessary training required to deliver and implement such hardware in synthetic biology laboratories (the Biomaker Challenge).

Milestones
K1: Annual funding round to support small scale innovative research projects.
Deliverable: Distribution of awards and public documentation of project results (annually, months 12-60, Haseloff, Osbourn).

K2: Annual support for open source hardware development and training (the Biomaker Challenge).

Deliverable: Co-sponsorship of student training, and development and documentation of open source hardware and bioinstrumentation (annually, months 12-60, Haseloff, Osbourn).

Progress to date
The milestones and deliverables for this work package are annual and progress has been on schedule.

K1: To date, the OpenPlant Fund has supported at total of 71 interdisciplinary and cross-institutional projects.

These projects are multidisciplinary, and the majority of them build new collaborations between Cambridge and Norwich, as well as with external partners including companies, institutes and universities from the UK and abroad. Projects range from DNA part development and testing, research method development, cell-free biology, open lab hardware, schools outreach, international capacity building, to IP policy, software and more. Half of all applicants are post-docs and over a third are PhD students. OpenPlant Fund projects have been very active in identifying routes to share their tangible outputs in the form of new technologies, DNA parts and an increasing number of publications.

In this last year of the OpenPlant grant, we have combined the OpenPlant Fund call with the Biomaker Challenge, as is described further under item **K2**.

Ad Hoc OpenPlant Fund awards:

Previous year, researchers from the University of Cambridge and the JR Biotek Foundation collaborated with researchers at the John Innes Centre and Earlham Institute to deliver a programme for a Molecular Biotechnology Laboratory Training Workshop attended by 20 African researchers from 8 different countries and culminating in the UK-Africa Food Security Symposium, addressing challenges and opportunities around partnership development and education in Africa. This year, the JR-Biotek team got awarded an additional £20.000 from OpenPlant (ad hoc award) to organize a second training workshop in Benin.

In addition, the Biomaker team that previously developed small scale speed breeding cabinets (with which they made it to the Finals of the BBRSC's Innovator of the year Awards), got awarded an additional £5000 to support a speed breeding workshop at the BecA-ILRI Hub in Nairobi, during which participants will build speed breeding cabinets that can stay at the Hub.

K2: The annual Biomaker Challenge is a four-month programme challenging interdisciplinary teams to build low-cost instruments for biology. This programme is coordinated by OpenPlant and the SynBio SRI, and funded by the former. Up to 50 grants are available to applicants from the University of Cambridge, John Innes Centre and Earlham Institute, though external partners are welcome. Successful applicants receive a Biomaker Starter Kit and a discretionary budget for additional sensors, components, consumables and 3D-printing worth up to £1000. At the end of the challenge, teams document their work on hackster.io, a publicly accessible platform, and exhibit their projects at the Biomaker Fayre as part of an Open Technology Workshop that celebrates frugal, open source and DIY approaches in research and education.

Outputs from the OpenPlant Fund and Biomaker Challenge projects are being published to the website www.biomaker.org. This website brings together information and outcomes of projects from the OpenPlant Fund, Biomaker Challenge and the previous Cambridge Synthetic Biology Fund.

The 2019 combined OpenPlant Fund/Biomaker call:

In this last year of the OpenPlant grant, we have combined the OpenPlant Fund call with the Biomaker Challenge, and offered teams an initial £750 plus a hardware package worth £250. After the first stage, teams could apply for an additional £2000 follow-on funding. We have had 27 diverse teams, of which most of them applied for and received follow-on funding:

- **Open DLS: an open-source dynamic light scattering device for nanoparticles sizing** (Etienne Rognin, Susannah Evans, Niamh Willis-Fox)
- **LunaFlow: Bioluminescent plankton for 3D flow visualisations of pressure fields** (Francesco Ciriello, Edoardo Gianni, Alessandra Luna Navarro, Duncan Scott, Nicholas Wise, Kayla Cervantes-Barron, Shivani Maharaj, Fernando Guzman-Chavez)
- **openCM - an Open Framework for Single Cell Manipulation** (Maziyar Jalaal, Nico Schramma, Stephanie Hohn, Kyriacos Leptos)
- **Stress priming for improved production of biotech-relevant compound in green alga** (Pawel Mikulski, Javier Santos Aberturas)
- **Mechanisms for direct electron transfer (DIET) between Geobacter and Methanothrix: possible effects of quantum mechanics and the role of membrane proteins** (Hannah Sanderson, Rohan Rao, Xinyue Wang)
- **Diabetes diagnosis and management using Arduino and mobile user interface** (Shalini Vaish, Varindra Kumar)
- **Developing an Open & Affordable 3D Bioprinter** (Sébastien Ricoult, Ben Porebski, Tejas Shah, Monica Saavedra, Robin Sterling, Julie Matte, Jia Wan)
- **CtoD: From Cells to Droplets** (Zhengao Di, Ziyi Yu)
- **e-CO-SENSE – Biophotovoltaic Powered Soil Sensors** (Vivek Badiani, Constantin Sahm, Glen Chua, Paolo Bombelli, Jaideep Prabhu)
- **An open toolkit for engineering microbial interactions** (Jarrod Shilts, Alex Baker, Georgeos Hardo, Kavi Shah)
- **Establishing a joint UK-Kenya Phytoplasma research initiative** (James Canham, Roland Wouters, Sylvain Capdevielle, Etienne de Villiers, Rose Kigathi, Florence Munguti, Maximilian Stammnitz, Lara Urban)
- **Build Your Own DNA Dave** (Sami Stebbings, Ioannis Tamvakis, Nadia Radzman, Jenni Rant)
- **Low Cost SLM Interface Board for Advanced Microscopy and Holographic Display of Biological Structures** (Andrew Kadis, Daoming Dong, Youchao Wang, Peter Christopher, Ralf Mouthaan)
- **SAFE - Safe Air for Everyone** (Victor Kang, Tiago Azevedo, Dushanth Seevaratnam)
- **Variable-time cameras with image recognition for inexpensive, large-scale monitoring of plant pollination events** (Roman Kellenberger, Carlos Lugo-Velez, Benjamin Kellenberger, Boris Delahaie, Allan Ellis)
- **Low Cost Oxygen Sensor for Bioreactors** (Chiara Gandini, Joseph Wong, Anne-Pia Marty)
- **MACRO-IMAGER: a low-cost multi-purpose large area macro digital photography phenotyping station** (Tobias Barber, Rowena Downie, Emily Marr, Lawrence Percival-Alwyn, Sam Mugford)
- **A behavioural chamber to evaluate rodent forelimb grasping performance** (Alejandro Carnicer-Lombarte, Ivan Dimov, Anastasios Polyravas, Damiano Barone)
- **BIOFAB INCUBATOR: Low cost programmable incubator for growing mycelium textile** (Catalina De Pablo Alday, Sebastian Rodriguez Jara, Claudia

Gaete Zúñiga, Guillermo Santiago Aviles Riquelme, Fernán Federici Noe, Jenny Molloy)
- **Aeroponics for All** (Jonathan Louis Kaplan, Fergus Riche, Alexandre Kabla)
- **Accupatch: Conductive microelectrode array for cancer tissue screening** (Saksham Sharma, Marta Wylot, Douglas Van Niekerk, Suraj Pavagada)
- **In-situ 3D visualization using X-ray CT during mechanical testing of natural cellular materials- like honeycombs and bones** (Angkur Jyoti Dipanka Shaikeea, Dr. Harika C. Tankasala, Malar Chellasivalingam)
- **BrewerMicro – DIY microscope for counting yeast** (Fernando Castro, Fernan Federici Noe, Alex Kutschera, Lawrence Percival-Alwyn, Sam Mugford)
- **IoHeat** (Erika Coletto, Dimitris Latousakis, Oscar Gonzalez, Andrea Telatin)
- **Did the organellar transit peptide duplicate itself to make a disordered linker protein?** (Indu Santhanagopalan, Vijaya Baskar)
- **Identification of genes involved in chloroplast division by comparison of temporal transcriptomics of *C. reinhardtii* and *A. thaliana*** (Sean R Stevenson, Indu Santhanagopalan)
- **Engineering Low-cost Turbidostat Systems for Running Microbial Evolution Experiments** (Somenath Bakshi, Soham Garg)

Evidence of the quality of the research

Publications:

Ghosh S, Watson A, Gonzalez-Navarro OE, Ramirez-Gonzalez R, Yanes L, Mendoza-Suárez M, Simmonds J, Wells R, Rayner T, Green P, Hafeez A, Hayta S, Melton RE, Steed A, Sarkar A, Carter J, Perkins L, Lord J, Tester M, Osbourn A, Moscou MJ, Nicholson P, Harwood W, Martin C, Domoney C, Uauy C, Hazard B, Wulff BBH, Hickey LT (**2018**) Speed breeding in growth chambers and glasshouses for crop breeding and model plant research. Nat Protoc. 13(12):2944-2963. doi: 10.1038/s41596-018-0072-z.

Other evidence of impact:

Gonzalez Navarro and colleagues (the speed breeding team) made it to the finals of the BBSRC Innovator of the Year Awards (https://www.jic.ac.uk/news/norwich-research-park-team-in-line-for-early-career-innovator-award/).

Workpackage L: OpenPlant Forum: responsible innovation

Relationship to other projects/themes
This workpackage spans all other workpackages in OpenPlant. The annual Forum meeting encourages attendance from all OpenPlant participants and OpenPlant Fund recipients, and all workpackages should be represented. SAW workshops are coordinated by Dr Jenni Rant and opportunities exist for all workpackages and OpenPlant Fund projects to interact.

Timing
Started September 2014 (Month 1)

Investigators
David Baulcombe (0.5 days), Dale Sanders (0.5 day), Jim Haseloff (8 days), Anne Osbourn (3 days)

Staff Employed
Jenny Molloy (OpenPlant Project Coordinator, Cambridge) February 2015 –February 2018
Colette Matthewman (OpenPlant Programme Manager, Norwich). Started October 2014 – Ended February 2019
Alexandra Ting (OpenPlant and SynBioSRI Communications Officer). Started January 2017 – Ended September 2019
Samantha Stebbings (Administrator). Started November 2017
Dieuwertje van der Does (OpenPlant Programme Manager, Norwich). Started February 2019

Partners
Dr Jenni Rant - The SAW Trust
Dr Linda Kahl – BioBricks Foundation
Dr Joanne Kamens - Addgene

Aims
This workpackage involves activities of the annual OpenPlant Forum, the annual working group and workshops and other public engagement activities with the SAW Trust. The OpenPlant Forum will provide a platform for exploring the potential applications of reprogrammed biological systems, and a framework for exploring the wider implications of the potentially disruptive new technologies. Each year, in association with the Forum, a working group will be established for in depth investigation of a topic relevant to the Forum theme.

The SAW Trust provides training in project design to scientists working in collaboration with professional artists and writers who come together as teams to deliver projects themed on the scientists' research topics.

Milestones
L1.1: Annual symposia on a series of themes related to plant synthetic biology
Deliverable: Devise and convene annual meetings (annually months 12-60, Haseloff, Osbourn).

L1.2: Recruitment of annual working groups
Deliverable: Appoint working groups around the symposia themes, with membership rotating to suit (annually, months 12-60, Haseloff, Osbourn, Baulcombe, Sanders).

L1.3: Annual published report
Deliverable: Document the themed output of the working group and symposium speakers (annually, months 12-60, Haseloff, Osbourn).

L2: SAW workshops

Deliverable: Co-sponsor SAW Trust workshops (annually, months 12-60, Osbourn).

Progress to date

L1.1: One of the key deliverables for addressing responsible innovation is the arrangement of an annual themed Forum to discuss wider questions in the field of plant synthetic biology. The 2019 OpenPlant Forum was themed "Smart design for the future Bioeconomy" and took place in Cambridge from 19-31 July. The Forum was coupled with the Biomaker/OpenPlant Fund pitches showcasing educational activities and resources developed through OpenPlant Fund and Biomaker Challenge projects (see Workpackage K).

L1.2: Covered in previous report.

L2: The OpenPlant partnership with the SAW Trust delivers large and important aspects of our ethical, social and policy programme. To date, we have delivered ten OpenPlant-themed workshops in primary schools, designed and delivered by research scientists in collaboration with SAW, influencing the educational approach to these topics. In the past year, we have also engaged with the public by delivering an inspiring interactive exhibit at the London Lates ("Year of the Engineer", Oct 2018).

SAW developed a training workshop to enable dissemination and to share best practice with other research centres. In November 2018, SAW ran a workshop session for "Talking Plants", the annual conference of the Botanic Gardens Education Network, hosted at Cambridge Botanic Gardens. Garden staff from across the UK's network came together to explore ways of bringing plant science to a wider audience.

Key Projects for Responsible Research and Innovation

1. The Global Garden workshop is a collaborative project with the SAW Trust and Social Scientist Dr Nick Lee (Warwick Integrative Synthetic Biology Centre). It invites people to co-learning workshops where they engage in discussion and exploration of views on global genetic plant resource sharing. The workshops begin with hands on science activities, including extracting DNA from strawberries, followed by discussion of real plant case studies and two further sessions where participants can share their personal creative response to plants, chemicals and people through art and poetry. We are preparing to run the next Global Gardens workshop at Cambridge Botanic Gardens in October 2019 as part of the city-wide Festival of Ideas. Places are bookable by members of the public but we also have a number of reserved places for local sixth form students.
2. In 2017, Dr Colette Matthewman and Dr Jenni Rant (SAW Trust) secured funding from the Biochemical Society to create a machine that explains the processes of transcription and translation in a fun and interactive way. They worked with artist Molly Barrett to develop DNA Dave the robot. We were awarded a small grant from the 2019 Biomaker Challenge (see workpackage K) to develop a 'Build your own DNA Dave' workshop for schools. We were also awarded follow-on funding from the Biochemical Society to continue our work with DNA Dave. Thus far, we have a DNA Dave design blueprint produced by a local engineering centre that will be incorporated into a resource pack for schools. In collaboration with Ioannis Tamvakis from the Sainsbury Laboratory Cambridge University (SLCU), we are translating the inner workings of DNA Dave from Arduino to BBC Micro:bit to enable schools to learn how to code and construct electronic components to represent biological processes in the robots they will build. Together with Nadia Radzman, also from SLCU we are creating a mini DNA Dave resource kit and workshop guide to trial with schools that we will be presenting at the Biomaker Fair in November 2019.

OpenPlant - SAW Primary School Workshops

1. In May 2019, SAW-style activities on the theme of plants designed by Samantha Stebbings were trialled at Chestnut Nursery with pre-school aged children. The children carouseled around activities to learn about different types of seeds, pollen, flower colour, plant growth and made their own vertical gardens.
2. In March 2019, Dr Jenni Rant and Samantha Stebbings delivered a SAW project of plants at West Earlham Junior School as part of their science week activities
3. In January 2019 a whole-school SAW activity was set up by Dr Jenni Rant at Cawston Primary School where topics such as DNA, cells and plant growth were explored.

OpenPlant at Science and Music Festivals

In October 2018 we worked with synthetic biologists from the University of East Anglia to design and deliver a stand for a 'Lates' event at the Science Museum in London. The theme of the stand was on natural products from plants and featured the avenacin pathway in oats which is a key research topic in the Osbourn lab. The visitors were typically aged between 18 – 45 years and were very interested to learn how bioinformatics can be used to mine genomes for genes in a pathway, how the HyperTrans system can be used to express genes to harness products and were even able to explore molecules using a VR headset.

Evidence of the quality of the research

Publications

Halewood M, Chiurugwi T, Sackville Hamilton R, Kurtz B, Marden E, Welch E, Michiels F, Mozafari J, Sabran M, Patron N, Kersey P, Bastow R, Dorius S, Dias S, McCouch S, Powell W (**2018**). Plant genetic resources for food and agriculture: opportunities and challenges emerging from the science and information technology revolution. New Phytol. 217(4):1407-1419. doi: 10.1111/nph.14993.

Workpackage M: Governance and Management

Relationship to other projects/themes

This work package is related to all themes in the centre as it involves coordination between all projects and groups and also outreach activities.

Investigators

David Baulcombe (3 days); Dale Sanders (3 days); Jim Haseloff (34 days); Anne Osbourn (7 days)

Staff Employed

Jenny Molloy (OpenPlant Project Coordinator, Cambridge) February 2015 –February 2018

Colette Matthewman (OpenPlant Programme Manager, Norwich). Started October 2014 – Ended Februari 2019

Alexandra Ting (OpenPlant and SynBioSRI Communications Officer). January 2017 – September 2019

Samantha Stebbings (Administrator). Started November 2017

Dieuwertje van der Does (OpenPlant Programme Manager, Norwich). Started February 2019

Partners

N/A

Aims

This workpackage is responsible for the overall management and coordination of the project. This involved key participants and coordinators from within the projects and external advisors. The Coordination and Management groups play the major role in monitoring progress, and contingency planning. This workpackage is responsible for running project management meetings, and ensuring coordination of activities between the Cambridge and Norwich sites. It is also responsible for coordinating the OpenPlant Forum and associated pump-priming and outreach activities.

Milestones

M1: Monthly meetings of the Coordination Group
Deliverable: Monthly reports to Management Group (months 1-60, Haseloff, Osbourn).

M2: Quarterly meetings of the Management Group
Deliverable: Quarterly progress review and report (quarterly, months 3-60, Haseloff, Osbourn, Baulcombe, Sanders).

M3: Annual meetings of the Advisory Board

Deliverable: Annual report of overall progress (annually, months 12-60, Haseloff, Osbourn, Baulcombe, Sanders).

M4: Organisation of annual All-Hands meetings
Deliverable: Presentations and web based documentation of workpackage efforts (annually, months 12-60, Haseloff, Osbourn).

M5: Management of the OpenPlant Fund
Deliverable: Annual summary of the allocation of funding for new projects and documentation of outcomes for existing projects (annually, months 12-60, Haseloff, Osbourn, Baulcombe, Sanders).

M6: Management of the OpenPlant Forum
Deliverable: Selection of annual theme for Forum, suggestions for invited speakers and review of costs (annually, months 12-60, Haseloff, Osbourn, Baulcombe, Sanders).

M7: Coordination of SAW activities
Deliverable: Report of co-funded SAW Trust activities, and identification of opportunities for participation by OpenPlant scientists (annually, months 12-60, Osbourn).

M8: Coordination of OpenLabTool activities
Deliverable: Report of co-funded OpenLabTools activities (annually, months 12-60, Haseloff).

Progress to date

M1: Apart from staff change (see above), ongoing and unchanged since previous report.

M2: Ongoing, and unchanged since previous report.

M3: The fifth Science Advisory Board Meeting took place on 31 July 2019 following the Annual OpenPlant Forum. The response of the SAB to the OpenPlant achievements discussed in the meeting was highly positive. This year discussions focussed mainly on opportunities for the future and the impact of the centre.

M4: Covered in previous report.

In addition, the OpenPlant Fund and Biomaker Challenge mixer events continue to provide great opportunities for exchange of ideas between researchers in Norwich and Cambridge. Moreover, we continue to share information via email and through the OpenPlant Newsletter and twitter.

M5: In this last year of the OpenPlant grant, we have combined the OpenPlant Fund call with the Biomaker Challenge, as described in workpackage K.

M6: The 2019 OpenPlant Forum was themed “Smart design for the future Bioeconomy” and took place in Cambridge from 19-31 July. The Forum was coupled with the Biomaker/OpenPlant Fund pitches showcasing educational activities and resources developed through OpenPlant Fund and Biomaker Challenge projects, as described in workpackage L.

M7: Unchanged since previous report. New outputs are described in workpackage L.

M8: OpenLabTools activities are delivered through the Biomaker Challenge. Excitingly, the Biomaker Challenge got awarded £80 k from GCRF for implementation of the programme in various African countries to stimulate local capacity building.

Evidence of the quality of the research

Covered in previous report.

LEADERSHIP AND MANAGEMENT

The management structure is unchanged from the original grant proposal, and is as outlined in previous annual reports. The Scientific Advisory Board is now chaired by Tom Knight, as mentioned in the previous report. Markus Gershater has taken the place of Tim Fell, who needed to withdraw due to work commitments.

TRAINING AND CAREER DEVELOPMENT

Covered in previous report.

ADDED VALUE

Covered in previous report.

IMPACT

Covered in previous report.