OpenPlant

Annual Report 2018

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

BBSRC bioscience for the future
EPSRC Pioneering research and skills
UNIVERSITY OF CAMBRIDGE
John Innes Centre
Earlham Institute

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BBSRC-EPSRC Synthetic Biology Research Centre

University of Cambridge, Cambridge

John Innes Centre & Earlham Institute, Norwich

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EXECUTIVE SUMMARY

Progress in Year 4

In our fourth year of operation (Sept 2017-2018), 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**. New aspects of the work include OpenPlant participation in publication of the *Marchantia polymorpha* genome sequence (7) and publication and launch of the OpenMTA (11), together with a series of significant advances in trait development (37-69), and expansion of the Biomaker programme (74).

Foundational work

1. Publication of quantitative microscale work showing intercellular adhesion promotes clonal mixing in growing bacterial populations.
2. Development of microfluidic vessels for quantitative observation of cyanobacteria.
3. Droplet-based microfluidic analysis and screening of single plant cells.
4. Construction of an improved generation of Loop Assembly-compatible transformation vectors for plants.
5. Establishment of efficient chloroplast transformation techniques in Marchantia.
6. Use of multispectral markers with *in planta* cytometry, to allow segmentation of cell geometries and quantitative assignment of biological parameters on a cell-by-cell basis.
7. Publication of the genome sequence and transcriptome datasets for Marchantia.
8. Publication of revision 4.0 of MarpoDB
9. Synthesis of an almost complete collection (350/398) of proximal promoters for transcription factors in Marchantia.
10. Collection of single-cell transcriptomic data from germinating gemma.
11. Launch of the OpenMTA license with a published commentary in Nature Biotechnology *36:923–927, 2018*.
12. Screening of cell and tissue type specific expression lines from enhancer trap transformants.
13. Development of a novel system for “resetting” Marchantia development by surgically dissecting gemma.
14. Miniaturisation and automation of efficient DNA Loop Assembly reactions using nanolitre scale handling.
15. Integration of Benchling as a free web-based solution for online lab notebooks, sequence editor and inventory handler.
16. Use of Benchling as a DNA registry and for protocol sharing across OpenPlant.
17. Construction of optimised genetic circuits as reporters for circadian clock output.
18. Identification of a number of putative electrically regulated promoters in cyanobacteria.
19. Characterisation of improved biophotovoltaic vessels and chassis strains.

20. Construction and publication of a MoClo kit, consisting in a set of Golden Gate-domesticated DNA parts, for the green alga *Chlamydomonas reinhardtii*.
21. High-throughput workflow to analyse conditional behavior of riboswitches *in vivo.*
22. MoClo parts for transformation of the chloroplast genome of *C. reinhardtii*.
23. Establishment of protocols to allow chloroplast transformation by electroporation in Chlamydomonas.
24. Regulation of chloroplast gene expression via TPR/PPR proteins encoded by the nucleus in Chlamydomonas.
25. Sequencing and reannotation of the chloroplast genome from the Cambridge isolates of *M. polymorpha*.
26. Construction of a new generation of Loop Assembly vectors for plastid transformation in Marchantia.
27. Optimisation of biolistic delivery protocols for high efficiency plastid transformation in Marchantia.
28. Generation of ftsz1 and ftsz2 mutant plant lines that produce fewer and larger plastids per cell.
29. Development of techniques and novel vector systems for RNA-guided Cas9-mediated targeted mutagenesis and gene deletion in plant species (Nicotiana, Arabidopsis, Marchantia).
30. Automated construct assembly and delivery to plant protoplasts to enable rapid quantitative assessments of the efficiency and specificity of gene editing.
31. Development of software-based 3-parameter measurement techniques for quantifying gene expression in cell suspensions to minimise extrinsic noise
32. Publication of a computational framework for automated analysis of microplate reader data.
33. Construction of models for the coupling of the cyanobacterial clock and control of period and cell growth.
34. Development of software-based classification schemes for description of gene expression at the cellular scale in Marchantia gemmae.
35. Maintenance and upgrades for the CellModeller software package, to include cell-cell adhesion and cell shape.
36. Publication of algorithms for whole colony-scale segmentation from confocal microscopy datasets of growing microbes.

Trait Development

37. Identification and functional characterisation of DNA parts for natural product synthesis.
38. Employment of genomic neighbourhood associations to identify triterpene-scaffolding genes with tailoring cytochrome P450s and acyltransferases.
39. Improvement of agro-infiltration methodology for gram-scale production of triterpenes using the HyperTrans transient plant expression system.
40. Publication of the improved infiltration protocol in text and video format.
41. *In silico* identification of new CYP450s with different oxidizing specificities.

42. Identification of new building blocks for biosynthesis of iridoids.
43. Discovery of neofunctionalisation of a short chain alcohol dehydrogenase (SDR) with a non-redox role in controlling the stereochemical course of ring cyclization during iridoid synthesis.
44. Blocked the turnover of L-DOPA in beetroot, using CRISPR/Cas9-mediated genome editing, to enable low-tech accessible production in a plant system.
45. Development of a novel suspension culture production system that produces exceptionally high levels of anthocyanins.
46. Over-expression of the Rosea1 and Delila transcription factors to produce multiply-acylated blue anthocyanins.
47. Activation of the anthocyanin biosynthetic pathway in cultures of *Arabidopsis thaliana* to produce diacylated cyanidin with a blue colour at neutral pH.
48. Identification and characterisation of transcription factors for control of natural product production.
49. Use of protocols for chromosome conformation capture and FISH analysis to investigate the three-dimensional positioning of biosynthetic gene clusters in the nucleus of *Arabidopsis thaliana*.
50. Identification of candidate transcription factors that regulate promoters of metabolic gene clusters including central pathway clusters and the avenacin pathway .
51. Use of a truncated version of the bHLH transcription factor Delila along with Rosea1 to fine-tune anthocyanin production for increased yield.
52. Testing of nine promoters from the oat avenacin cluster to demonstrate retention of characteristic expression patterns when introduced into diverse plant species as promoter-reporter constructs.
53. Three promoters from the oat avenacin cluster were used to successfully drive the expression of a three-gene pathway for a plant defence compound (dhurrin) from sorghum in *Arabidopsis thaliana* roots.
54. Development of robust methods for co-cultivation of Marchantia spp. with Glomeromycota fungi and visualisation of colonization.
55. Development of a high-throughput transformation system for *Marchantia paleacea*, with marker systems for labelling the secretory system and tonoplast.
56. Establishment of reproducible colonisation of several liverwort species (*Marchantia spp*., *Lunularia cruciata*) with Glomeromycota fungi (*Funnelliformis mossae*, *Rhizophagus irregularis*).
57. Measurement of the Marchantia transcriptome in response to filamentous pathogen colonisation to identify a set of genes strongly upregulated during defense.
58. Identification of the MYB14 transcription factor that produces a red pigment in cells, useful for sector analysis.
59. Production of a draft genome sequence for *M. paleacea* using paired end libraries and illumina sequencing.
60. The three main symbiosis TFs in (IPD3, NSP1 and NSP2) in *M. paleacea* were tested in complementation assays in Medicago mutants.

61. Integration of Marchantia as a model system in the Bill and Melinda Gates Foundation Engineering Nitrogen Symbiosis for Africa project, which was recently awarded Phase 2 funding.
62. Analysis of transcriptome data to indicate that a Marchantia syntaxin (SYP13B) whose legume ortholog is implicated in nitrogen fixing symbiosis may also play a role in intracellular colonisation by an oomycete pathogen.
63. Development of new Hyper-Trans vectors to enable fine-tuning of protein expression levels by making changes in the 5'- and 3'-UTRs of the CPMV-HT system
64. Construction of modified components of the CPMV-HT system that are compatible with the OpenPlant plant common syntax.
65. Development of a new synthetic version of the 5' UTR used in the HT system, which is twice as effective as the original.
66. Two new vectors (pHRE and pHREAC) have been finalised and are ready for distribution.
67. 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).
68. The CPMV-HT system has been translated to the BY2 cell pack system, and is also being used in tomatoes and tested in Marchantia.
69. Successful expression of a protein from *Theileria parva* (causal agent of East Coast Fever, a veterinary disease in Sub-Saharan Africa) which has been difficult to express in other systems.

Outreach and Responsible Innovation

70. Jenny Molloy awarded Shuttleworth Fellowship to explore impact of open technologies on innovation in biology at an international scale.
71. The OpenPlant Fund has funded 19 new projects over the last year, to bring the total to 71 interdisciplinary and cross-institute projects.
72. Organisation of cell-free hands-on workshops to encourage the development of cell-free technologies through the OpenPlant Fund
73. Initiated two projects working with African researchers, with JR Biotek Foundation Workshop in Cambridge, attended by 20 African researchers from 8 different countries and culminating in the UK-Africa Food Security Symposium, and working with the Kumasi Hive and Lab_13 project in Ghana to develop resources for teaching synthetic biology in low-resource settings.
74. Organised the second annual Biomaker Challenge for interdisciplinary teams to build low-cost instruments for biology. In two years, we have funded 61 projects involving close to 200 participants from Cambridge, Norwich and beyond.
75. Implementation of a 5-session training series aimed at teaching basic hardware and software for scientists.

76. Adopted the visual programming interface XOD to tackled some of the bottlenecks and difficulties that non-programmers face when building electronics.
77. Publication of tutorial material at www.biomaker.org.
78. Establishment of the Norwich Biomakers group to bring together an interdisciplinary network interested in biology, design, technology, engineering, electronics, software, art and more – to gain a membership of 180 members in the first year.
79. Ran ten Norwich Biomaker sessions over the first year. The network has led to several new collaborations.
80. The 2018 OpenPlant Forum focused on engineering plants for bioproduction, and explored a range of examples of products that can be made in plants, tools for engineering for bioproduction and both the global challenges and opportunities for harnessing biodiversity.
81. The Forum was coupled with the OpenPlant Fund pitches, and a Curriculum Hacks event showcasing educational activities and resources developed through OpenPlant Fund and Biomaker Challenge projects, as well as by partners such as Science and Plants for Schools (SAPS), Cambridge Biomakespace, and the University of East Anglia.
82. A new working group was established to investigate new models for documentation, distribution and publication by bioengineers - in particular focusing on documentation and distribution of characterisation data for DNA parts.
83. We have delivered seven OpenPlant-themed workshops in primary schools, designed and delivered by research scientists in collaboration with the SAW Trust.
84. We have engaged with the public by delivering interactive exhibits at the Norwich Science Festival and Cambridge Science Festival, and delivered science activities at Kidztown at the Boomtown Fair music festival and Latitude mixed arts Festival.
85. SAW developed training workshops to enable dissemination and to share best practice with other research centres, SynthSys and the UK Centre for Mammalian Synthetic Biology (University of Edinburgh), and the Warwick Integrative Synthetic Biology Centre.
86. An International conference established by the SAW Trust in collaboration with Norfolk County Council brought together education specialists from across the world locally and internationally to share learning platforms and develop ideas, at which SAW presented its school projects with OpenPlant.
87. Ran the Global Garden workshop, a collaborative project with the SAW Trust and Social Scientist Dr Nick Lee (Warwick Integrative Synthetic Biology Centre).
88. Secured funding from the Biochemical Society to create and exhibit a machine that explains the processes of transcription and translation in a fun and interactive way - DNA Dave the robot.

INTRODUCTION

Plants are attractive platforms for synthetic biology and metabolic engineering. Their modular and plastic body plans, capacity for photosynthesis, extensive secondary metabolism, and agronomic systems for large scale production make plants ideal targets for genetic reprogramming. However, efforts in this area have been constrained by slow growth, long life cycles, the requirement for specialized facilities, a paucity of efficient tools for genetic manipulation, and the complexity of multicellularity. There is a need for better experimental and theoretical frameworks to understand the way genetic networks, cellular populations and tissue-wide physical processes interact at different scales.

New biological techniques are offering radical approaches to the analysis and large-scale synthesis of genetic systems in plants. This is part of a technical revolution in our ability to construct DNA code and to edit existing genomes in order to reprogramme the growth and physiology of organisms. When applied to plant systems, these new synthetic biology technologies offer the long-term prospect of rational design and programming of new plant traits, with potentially revolutionary consequences for agriculture and bioproduction.

Over the last century, agriculture has become increasingly intensive and industrialised. Crop farming in the developed world is highly mechanised, with ever-increasing reliance on fertilisers and pesticides and highly tuned and efficient germplasm. Innovations in agriculture have led to an explosive growth in the extent of protected technologies and plant varieties at play in the field. At the same time, we are seeing an unparalleled consolidation of ownership, as large corporations merge and integrate their agrochemical and seed businesses. With the mergers of Dupont-Dow, Monsanto-Bayer and Adama-Syngenta, a handful of companies will share a 70-80% stake in global seed and agrochemical sales. The last century has seen a growing intensification of agricultural practices, with increased adoption of vertically integrated agribusiness models, closed technologies and restrictive licensing practices in the field.

New technologies offer the prospect of a breakout from the limitations of conventional breeding and the single-gene GM agronomic traits that currently dominate the market. However, the new approaches require access to multiple DNA elements, sophisticated design and assembly tools, multi-scale analyses for debugging, and above all, sharing of knowledge. Synthetic Biology offers the prospect of rational design and reprogramming of biological systems for improved and sustainable bioproduction. While early efforts in the field have been directed at microbes, the engineering of plant systems provides even greater potential benefits. In contrast to microbes, plants are already globally cultivated at extremely low cost, harvested on the giga-tonne scale, and routinely used to produce the widest range of biostuffs, from fibres, wood, oils, sugar, fine chemicals, drugs to food. Plants are genetically facile, and GM plants are currently grown on the >100 million hectare scale. Plant systems are ripe for synthetic biology, and any improvement in the ability to reprogram metabolic pathways or plant architecture will have far-reaching consequences.

We believe that there is a clear opportunity and need to create open systems and free tools for biological engineering in plants. The availability of open systems will create a self-reinforcing

ecosystem for exchange of scientific knowledge and resources, and will facilitate innovation in research laboratories. Frameworks for the easy commercial use of low-level tools will promote entrepreneurship and social enterprise. The technology is not intrinsically expensive. Open frameworks for education and sharing would facilitate technical transfer to developing countries - which are often rich in biological resources, but lack the capacity to take full advantage of their potential. OpenPlant aims to create open frameworks for sharing that will facilitate equitable exchange of knowledge, innovation and enterprise for the new biotechnologies that will be crucial for global food security and future sustainable production.

OpenPlant is a joint initiative between the University of Cambridge, the John Innes Centre and the Earlham Institute, funded by the BBSRC and EPSRC as part of the UK Synthetic Biology for Growth programme. The initiative promotes (i) interdisciplinary exchange, (ii) open technologies and (iii) responsible innovation for improvement of sustainable agriculture and conservation.

The UK provides an ideal hub for interdisciplinary exchange between foundational sciences like botany, agronomy, physics, chemistry, computer sciences and engineering. This exchange drives innovation for the engineering of biological systems. OpenPlant promotes and funds the development of novel foundational technologies, the creation of international standards for plant synthetic biology, and open tools for trait development. We believe that advances in plant synthetic biology will provide a key to securing and sustaining future food and materials production, and that there should be worldwide open access to these benefits.

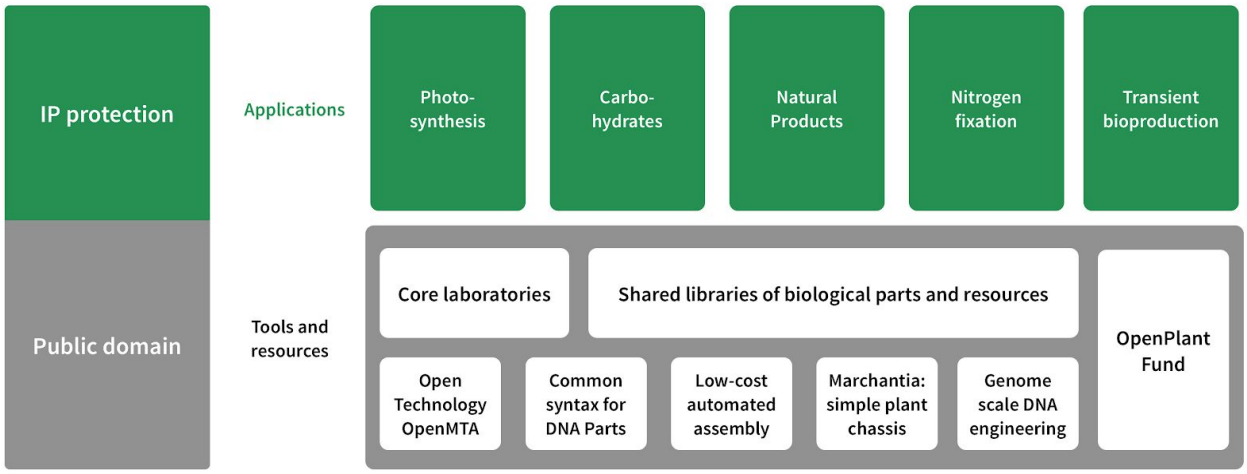
Current IP practices and restrictive licensing threaten to restrict innovation as the scale of DNA systems increases. We believe that the field needs to explore new “two-tier” intellectual property models that will protect investment in applications, while promoting sharing of DNA components and freedom-to-operate for innovators in business and social enterprises. We are building new frameworks and collaborations for open innovation in plant synthetic biology.

The OpenPlant initiative has been funded with three main aims:

1. to create a hub for interdisciplinary exchange between Cambridge and Norwich, between the fundamental and applied sciences, that will underpin advances in UK agriculture and bioproduction.
2. to establish systems for the open exchange of new plant tools and DNA components that will promote commercial innovation and international scientific exchange.
3. to explore the wider implications of the technology at local and global scales. This will bring together a wide range of engineers, scientists and policy developers to explore new technologies and possible models for sustainable agriculture, bioproduction and land use.

WORKPACKAGES

The OpenPlant initiative supports tiers of activities, (i) development of open standards and shared resources for plant engineering, (ii) prototyping of new crop traits, and (iii) promotion of exchange, training and innovation.



(i) Open Standards and Engineering:

Development of open standards, resources and technologies for engineering plant systems.

Workpackage A: Development of the lower plant Marchantia as a highly simplified and facile chassis for reprogramming plant form and physiology.

Workpackage B: A common syntax for plant DNA parts and assembly of genetic circuits. Establishment of a shared library of DNA parts for plant engineering.

Workpackage C: New DNA parts for the control and quantitative imaging of genetic circuits.

Workpackage D: Techniques for routine genome-scale engineering in plants.

Workpackage E: Software tools with improved performance for DNA part catalogues, automated assembly, and modelling of synthetic gene circuits and cellular morphogenesis.

(ii) Plant Trait Engineering:

Application of the new ways of working to engineer target traits in plants.

Workpackage F: Altered photosynthesis and leaf structure.

Workpackage G: Changes in plant carbohydrate content.

Workpackage H: Engineered pathways for the metabolic engineering of natural products.

Workpackage I: New forms of symbiosis and nitrogen fixation for crop plants.

Workpackage J: Methods for high level production of biomolecules by transient expression.

(iii) Shared Innovation:

Promotion of global access and interdisciplinary training for equitable sharing and innovation.

Workpackage K: Mini-funds to seed novel interdisciplinary exchange and innovation.

Workpackage L: Outreach activities, training and tools for open exchange of DNA parts and other reagents in biotechnology.

Workpackage M: Project management and communication

VISION AND AMBITION

As part of the OpenPlant initiative, we plan to implement technologies for engineering plants:

- Open DNA registries for sharing information, and access to standardised plant specific parts.
- A major new plant chassis for Synthetic Biology, with simple properties for high throughput screening and analysis at the micron scale.
- New DNA parts for the control and quantitative imaging of genetic circuits in plants.
- Techniques for routine genome-scale engineering in plants.
- Software tools with improved performance for automated DNA assembly, modeling of synthetic gene circuits and cellular morphogenesis.

The development of new tools and parts will directly contribute to the engineering of new traits in plants, such as:

- Altered photosynthesis and leaf structure.
- Changes in plant carbohydrate content.
- Engineered pathways for the metabolic engineering of natural products.
- New forms of symbiosis and nitrogen fixation for crop plants.
- Methods for high level production of biomolecules by transient expression.

Current agricultural practices and cultivation of trees, crops and pastures are responsible for major pressures on natural environments and land use globally. The OpenPlant initiative brings together an exceptional collection of scientists, whose skill sets range from biophysics, chemistry and DNA assembly - to crop physiology and agronomy. In addition, we have recruited experts involved in conservation, entrepreneurship, law, policy development and the social sciences in Cambridge and elsewhere in the UK – who have demonstrated an interest in tackling the technical aspects of surveying future technologies. An overarching aim of the project is to provide a map of feasible technical approaches to improving bioproduction and agriculture – including studies of possible economic models, opportunities and social implications for different scenarios and current practices.

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.

Current:

Susana Sauret-Gueto (Research Manager; Haseloff lab). Started October 2015.
Linda Silvestri (Research technician, Haseloff lab). Started February 2016.Eftychios Frangedakis (PDRA, Haseloff lab) Started April 2017
Marta Tomaselli (OpenPlant PhD student). Started April 2017

Partners

Bernardo Pollak; Christian Boehm; Mihails Delmans, Marius Rebmman

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 (2017-2018)
A2: Tim Rudge was appointed with OpenPlant funds to work with Pietro Cicuta in the Cavendish Laboratory, to finalise microscale approaches for quantitative measurement of gene expression in single cells. Using E. coli expressing GFP under the control of both constitutive and regulated promoters, he investigated correlations between the rate of growth and the rate of gene expression, and the spatial variations of both of these within the colony. It was possible to optimise confocal imaging of colonies growing from single cells up to millimetre size three dimensional colonies. Initial results indicate changes in both growth and gene expression between the core and edges of the colony, and image analysis, including optical flow algorithms was used to extract growth when cell segmentation became unfeasible. This work provides programmable cell populations as testbeds, and allows better understanding of how physical structure in a growing clonal population can lead to morphological differentiation even in a simple prokaryote system. The work has led to several publications, including work led by Anton Kan (Haseloff lab) and recently published in the Royal Society Interface journal (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: 20180406. http://dx.doi.org/10.1098/rsif.2018.0406).

This microscale approach has been applied to development of microfluidic vessels for quantitative observation of cyanobacteria in the the-green-mother-machine-reloaded Openplant Fund project by Bruno Martins and colleagues), and to plant cells. The latter work was recently published in Yu Z, Boehm CR, Hibberd JM, Abell C, Haseloff J, Burgess SJ, Reyna-Llorens I (2018). Droplet-based microfluidic analysis and screening of single plant cells. PLoS One. 2018 13:e0196810. doi: 10.1371/journal.pone.0196810.

A3: Marchantia transformation systems are well established. The spore transformation protocol is widely used by OpenPlant groups, and is continuing to be refined, for example through miniaturisation and scale-up in numbers of transformants. We have created a second generation Loop Assembly-compatible transformation vectors, and are testing new promoters and herbicide resistance markers for *in planta* selection. Dr. Susana Sauret-Gueto (Haseloff lab) is coordinating this project and interacting with others in the Marchantia community, including Suvi Honkanen from the University of Western Australia, and researchers in the Schornack lab. We use fluorescent markers to screen for successful transformants, which allows identification around 5 days after plating the transformed sporelings, and plant transfer by 2 weeks.

A major achievement over the last year has been the establishment of efficient chloroplast transformation techniques by Kasey Markel and Dr. Eftychios Frangedakis (Haseloff lab). This opens up a new avenue for research, as chloroplasts have great potential as novel, low cost vehicles for bioproduction and are capable of producing prodigious amounts of product. In addition they have optimised thallus transformation, which allows super-transformation of mutants or marker lines without need for spore production (which is a lengthy 2-3 month process). Sauret-Gueto has compiled protocols for Marchantia work, being documented at protocols.io and the website www.marchantia.org that she curates. She has also established the ROC group (Researchers with OpenPlant in Cambridge) with members from different labs/Departments interested in sharing resources for Synthetic Biology. A Marchantia-ROC subgroup meets monthly and is a key group for sharing knowledge of Marchantia protocols, promoting type IIS assembly, and synthesis and characterisation of standardised DNA parts for Marchantia.

A4: As reported last year, Bernardo Pollak and Linda Silvestri (Haseloff lab) have established a reliable agar-trap Marchantia spore transformation system, which is capable of high throughput production of transgenic plants. We have refactored spectral variants of fluorescent proteins for efficient expression in Marchantia. A number of these have been published (Mihails Delmans*, Bernardo Pollak* and Jim Haseloff. Plant Cell Physiol. 58: e5(1–9) 2016). These co-localised multispectral markers are suitable for use in our *in planta* quantitative cytometry method (Federici, et al. Nature Methods, 9:483-485 (2012). The vectors contain spectrally distinct fluorescent protein markers that allow autosegmentation of cell geometries and quantitative assignment of biological parameters on a cell-by-cell basis. The Haseloff lab is continuing to improve the system, testing multiple combinations of promoters, fluorescent proteins and signal peptides, and employing vectors compatible with Loop assembly.

A5: Draft genome and transcriptome sequences for the CAM-1 (male) and Cam-2 (female) isolates of *Marchantia polymorpha* have been produced in the Haseloff lab, and were published last year in Cell, as part of a community effort to characterise the Marchantia genome (JL Bowman, T Kohchi, KT Yamato, J Jenkins, S Shu, K Ishizaki, et al. (including Pollack B., Delmans M, Boehm CR, Haseloff J) Cell 171 (2), 287-304. E15, 2017). Polymorphisms between the Tak-1 and Cam-1/2 isolates are around 1/1000 bp within genes. In addition, the Oldroyd lab has generated genome sequence for M. paleacea (which, unlike *M. polymorpha*, forms symbiotic fungal associations). Mihails Delmans continued to improve the OpenPlant MarpoDB,

the gene-centric database (www.MarpoDB.io) that allows facile access to gene features from the *M. polymorpha* genome. Version 4 of the database was released in August 2018) added the 3.1 version of the Marchantia Tak-1 reference genome. The annotation has been recompiled with the latest version of InterPro and Uniprot, and gene names were aligned with the community reference at MarpoIBase (marchantia.org).

MarpoDB provides features that allow mining of the genome dataset for synthetic promoters, genes and terminators that can be exported directly as sequence files for synthesis of standardised Phytobrick DNA parts. An almost complete collection of proximal promoters for transcription factors (TF) in Marchantia (350/398) have been refactored, chemically synthesised and cloned as standard L0 parts for Loop assembly. Dr. Sauret-Gueto established a registry for L0 parts using Benchling and is curating the database. Linda Silvestri is maintaining the stocks of L0 parts. In parallel with this effort, we have built Loop assembly compatible vectors that are compatible with the OpenMTA, and thus suitable for open distribution. All L0 parts have been cloned into these new vectors.

In September 2018, we received a pre-publication copy of version 4 of the Marchantia Tak-1 genome. This assembly is the result of collaborative efforts, led by Fred Berger, Vienna - to generate entire chromosomal assemblies through the use of Pac-Bio sequencing and Hi-C mapping. We now have chromosome-scale contigs and are using this data to assemble full alignments of the Cam-1 and Cam-2 genomes. This data allows a confident assignment of the full gene complement in Marchantia, and a well defined target for development of DNA tools and genetic reprogramming.

A6.1: The assembly of an annotated Marchantia genome has allowed precise analysis of transcription dynamics, using RNAseq studies of germinated Marchantia spores. Changes in mRNA production were mapped over the crucial early stages of germination, cell enlargement, chloroplast differentiation, asymmetric cell division, rhizoid and thallus formation and cell differentiation. This data was published in the recent Marchantia genome paper (Cell 171:287-304, 2017). The resultant data has been used to evaluate expression of transcription factors and metabolic markers. Further Mihails Delmans has collected single-cell transcriptomic data from germinating gemma. With Marius Rebmann (Haseloff lab), they have collected and classified the range of cell types found in these tissues. They are now validating these RNA fingerprints by studying the cell-specific expression of a range of markers.

A6.2: We are screening the family of TF promoters for cell and tissue specific expression patterns. The project is being coordinated by Dr. Susana Sauret-Gueto and Linda Silvestri, with the assistance of summer students and undergraduate project students. We will make those lines, as well as the L0 parts with the promoters, freely available to the community under the OpenMTA license (now launched with a published commentary in Nature Biotechnology *36:923–927, 2018*).

Another source of cell and tissue type specific expression lines comes from the screening of enhancer trap lines being generated in the Haseloff lab. This project is also being coordinated

by Silvestri and Sauret-Gueto, with assistance from undergraduate students. Marta Tomaselli is further characterising lines with air chamber related expression patterns and identifying genes and regulatory sequences that are responsible for key expression patterns.

A number of the promoter fusion and enhancer-trap markers are expressed early during gemma development and patterns of expression are being documented, especially for association with meristem, air chamber or rhizoid development. These form the basis of student projects to map and study cellular dynamics in this accessible system. Mihails Delamns has established a system for surgically dissecting Marchantia gemma. Removal of the meristematic tissue causes a “reset” of cell identities in the central part of the gemma, resulting in a burst of synchronous cell divisions, dynamic self-organised patterns of gene expression and regeneration of meristematic zones. This provides a unique and powerful system for analysing genetic and cellular organisation by direct quantitative imaging. We will use this system to analyse the new families of markers.

Evidence of the quality of the research

2017-2018 Publications:

Bernardo Pollak, Ariel Cerda, Mihails Delmans, Simón Álamos, Tomás Moyano, Anthony West, Rodrigo A. Gutiérrez, Nicola Patron, Fernán Federici & Jim Haseloff (2018). **Loop Assembly: a simple and open system for recursive fabrication of DNA circuits. New Phytologist**, *accepted for publication*.

Linda Kahl, Jennifer Molloy, Nicola Patron, Colette Matthewman, Jim Haseloff, David Grewal, Richard Johnson & Drew Endy (2018). **Opening options for material transfer**. *Nature Biotechnology 36:923–927.*

Christian R. Boehm, Bernardo Pollak, Nuri Purswani, Nicola Patron and Jim Haseloff (2017). ***Synthetic Botany***. Cold Spring Harbor Perspectives in Biology, doi: 10.1101/cshperspect.a023887.

Bowman JL, Kohchi T, Yamato KT, Jenkins J, Shu S, Ishizaki K, Yamaoka S, Nishihama R, Nakamura Y, Berger F, Adam C, Aki SS, Althoff F, Araki T, Arteaga-Vazquez MA, Balasubramanian S, Barry K, Bauer D, Boehm CR, Briginshaw L, Caballero-Perez J, Catarino B, Chen F, Chiyoda S, Chovatia M, Davies KM, Delmans M, Demura T, Dierschke T, Dolan L, Dorantes-Acosta AE, Eklund DM, Florent SN, Flores-Sandoval E, Fujiyama A, Fukuzawa H, Galik B, Grimanelli D, Grimwood J, Grossniklaus U, Hamada T, Haseloff J, Hetherington AJ, Higo A, Hirakawa Y, Hundley HN, Ikeda Y, Inoue K, Inoue SI, Ishida S, Jia Q, Kakita M, Kanazawa T, Kawai Y, Kawashima T, Kennedy M, Kinose K, Kinoshita T, Kohara Y, Koide E, Komatsu K, Kopischke S, Kubo M, Kyozuka J, Lagercrantz U, Lin SS, Lindquist E, Lipzen AM, Lu CW, De Luna E, Martienssen RA, Minamino N, Mizutani M, Mizutani M, Mochizuki N, Monte I, Mosher R, Nagasaki H, Nakagami H, Naramoto S, Nishitani K, Ohtani M, Okamoto T, Okumura M, Phillips J, Pollak B, Reinders A, Rövekamp M, Sano R, Sawa S, Schmid MW, Shirakawa M, Solano R, Spunde A, Suetsugu N, Sugano S, Sugiyama A, Sun R, Suzuki Y, Takenaka M, Takezawa D, Tomogane H, Tsuzuki M, Ueda T, Umeda M, Ward JM, Watanabe Y, Yazaki K, Yokoyama R, Yoshitake Y, Yotsui I, Zachgo S, Schmutz J (2017). **Insights into**

Land Plant Evolution Garnered from the Marchantia polymorpha Genome. *Cell 171(2):287-304.e15. doi: 10.1016/j.cell.2017.09.030*

Boehm CR, Pollak B, Purswani N, Patron N, and Haseloff J (2017). **Synthetic Botany.** *Cold Spring Harbor Perspectives in Biology, doi: 10.1101/cshperspect.a023887*

Other evidence of impact

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

Invited presentations (Sept 2017-2018)

International Plant Systems Biology Meeting, Roscoff, France. (10-14 Sept 2018), invited speaker (Haseloff).
EMBO workshop: New shores in Land Plant Evolution, Lisbon, Portugal (20-23 June 2018), invited speaker (Haseloff).
Computer Sciences Seminar, Bahir Dar University, Ethiopia (27th Mar 2018), invited seminar (Haseloff).
Synthetic Biology Society, Oxford University (31st Feb 2018), invited seminar (Haseloff).
Synthetic Biology Australasia Annual Meeting, Sydney, Australia (20-22 Sept 2017), invited speaker (Haseloff)
Synthetic Biology: Future Science Platform, Sydney, Australia (19-20 Sept 2017), invited participant (Haseloff).
ARM Summit, Cambridge, UK (13th Sept 2018), invited speaker (Haseloff).
EditBio 2017, Irapuato, Mexico (19-23 August 2017), 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
B1-2: We have established a common genetic syntax for exchange of DNA parts for plants, extensible to all eukaryotes (Patron et al. 2015). The syntax is compatible with range of existing techniques such as Gibson assembly, MoClo and Golden Braid, and can be used for higher order multiple-gene assemblies. However we have developed a simple and flexible protocol for assembly of plant vectors, the Loop Assembly technique. A description of the Loop Assembly method has been accepted for publication at New Phytologist.

As part of a collaboration between the University of Cambridge and the Universidad Católica de Chile, Federici and Pollak (Haseloff Lab) have devised a new method for recursive gene assembly based on two Type IIS restriction endonucleases, BsaI and SapI. Loop Assembly allows rapid and efficient production of large DNA constructs, is compatible with widely used Level zero (L0) DNA parts such as Phytobricks, and has been automated through collaboration with the DNA Foundry at the Earlham Institute.

The Patron Lab has miniaturized and automated DNA Assembly reactions at the Earlham DNA foundry (Patron Lab) to provide assembles for Workpackage D and B. Oleg Raitskin (Patron Lab) and colleagues at the Earlham Institute have automated assembly in <500 nL in 384 plates using the Labcyte Echo. Transformed bacteria are dispensed by shaking and multiple droplet dispensing onto agar in square, 8-well plates. Colony picking is performed on the Hamilton platforms, which have an on-board camera and light table. The EasyPic software is used to select positive colonies. Assemblies are validated using a Miniaturised Nextera XT library

construction on an Illumina MiSeq. Transfection of plant protoplasts using a modified isolation procedure with automated transformation on the Hamilton platform is ongoing.

As the project has progressed, we have developed MarpoDB, a customised gene-centric database for Marchantia that allows description of the genome as a series of DNA encoded parts, and allows easy refactoring and extraction of parts in standardised format for synthesis and reuse. Further, we have found that Benchling provides a free web-based solution for an online lab notebook, sequence editor and registry of entries (www.benchling.com). In Benchling, members of an organisation can share a registry, and a registry of Marchantia DNA parts has been established. Sauret-Gueto is in contact with the Benchling team to implement subgroups within an organisation, thus allowing other labs to use parallel registries with the same schemas (with pre-established fields to fill in the metadata), easier sharing of registries within OpenPlant, and public sharing of the registry. This combination of web-accessible databases is proving to be a more accessible and flexible solution as a DNA registry, and beginning to find wider use across and beyond the OpenPlant community.

We have documented synthetic DNA parts, and vectors and plasmids constructs on the Benchling web platform. We are using Benchling as a DNA registry and for protocol sharing across OpenPlant and have paired this with the use of Addgene as the main repository for DNAs.

B3: The Locke lab has constructed rationally designed DNA parts for examining the circadian clock and its outputs at the single cell level in the model cyanobacteria Synechococcus elongatus 7942. They have developed improved fluorescent reporters to enable better quantification of the output of the inducible promoters. The parts include codon optimised versions of newly available YFP and CFP fluorescent proteins as reporters for the key circadian (24 hour) clock output genes sigC and psbA1. The reporters contain a degradation tagged YFP to allow the analysis of fast changing gene expression dynamics. The reporters were used to examine psbA1 dynamics at the single cell level, and revealed that psbA1 displays 12 hour oscillations, and not 24 hour as previously thought. They went on to show that the frequency of the circadian clock in cyanobacteria can be doubled through an incoherent feedforward loop circuit involving sigC.

The lab has gone on to produce a further array of optimised reporters, with a range of degradation tag strengths. This allows the probing of a range of strength of transcriptional dynamics. The lab is further characterising these parts, with the aim of writing up a methods paper on the new resource They have also carefully characterised how the circadian clock modulates cell size and division in cyanobacteria. The understanding of this will be critical for building robust synthetic circuits that can withstand the daily oscillations in cell growth and size that cyanobacteria are subjected to via the environment and the circadian clock. A description of this work is under revision for publication in *PNAS*.

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. 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. Golden Gate Assembly is being used in accordance with the OpenPlant common syntax to construct variants of promoter regions that control a codon optimized mOrange2 gene. The importance of the cis-element, and the minimal region required for regulation, is being ascertained through the construction of multiple promoter variants. 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.

B5: A primary aim of the OpenPlant initiative is to establish and promote better practices for producing and sharing standardised DNA parts for engineering plant systems. To this end, we have made major advances:

Common syntax for the exchange of modular DNA parts. The syntax is a regularised and consistent version of that used in Golden Gate, MoClo, Golden Braid and ENSA Type IIS based DNA assembly methods, and was published as a technical manifesto with wide support from the international plant science community (Patron et al. New Phytol. 2015). We have also promoted the common syntax for plant DNA parts as a new standard for the iGEM competition (RFC 106, Rutten et al. 2015; http://hdl.handle.net/1721.1/96069). This was a collaborative output from the Cambridge-JIC, Valencia and Norwich Research Park iGEM teams. Following this, the iGEM foundation agreed that parts can be submitted to the Registry of Biological Parts in RFC 106 (and are termed Phytobricks). This set the foundation for an inaugural Plant Prize at the 2016 iGEM competition (won by Cambridge-JIC in 2016).

Open materials transfer. Current IP practices and restrictive licensing threaten to restrict innovation as the scale of DNA systems increases. We believe that the field needs to explore new "two-tier" intellectual property models that will protect investment in applications, while promote sharing of DNA components and freedom-to-operate for small companies in

commercial applications of Synthetic Biology. We are collaborating with Dr. Linda Kahl and colleagues at the Biobricks Foundation to draft and implement an Open Materials Transfer Agreement (OpenMTA). This is a simple, standardized legal tool that enables individuals and organizations to share their materials and associated data on an open basis. Materials can be freely used (even for commercial purposes) and further distributed by the recipients. The primary purpose of the OMTA is to effectively place low-level DNA parts into the public domain, and to eliminate or reduce transaction costs associated with access, use, modification, and redistribution of materials and associated data. This in turn will help minimize delay and redundancy in the scientific research process and promote access to materials and associated data for researchers in less privileged institutions and world regions. Details of the draft can be found at http://www.openmta.org, and a description has been published in Nature Biotechnology.

Distribution. In order to avoid unwanted restrictions on OpenMTA-based distribution, we have constructed, a "clean" plasmid vector (pUAP1) suitable for cloning of Level zero DNA parts. Further, we have constructed two families of LOOP plant transformation vectors, based on pCambia and pGreen backbones, respectively. These differ in replication origins and copy number in bacterial hosts. We have now generated a growing library of Marchantia TF promoters, as well as a basic Marchanta DNA tool-kit for core promoters, resistance genes, fluorescent proteins, signal peptides, CRISPR-Cas9 and other tools for plant synthetic biology. We are in discussions with Addgene - and the current plan is for adoption of the OpenMTA as an alternative to the standard UB-MTA for routine, open distribution of DNAs intended for the public domain at Addgene. This requires modification of the automated system at Addgene, and is expected by early 2019. We are keen to facilitate this, which would lead to global adoption of the OpenMTA.

Evidence of the quality of the research

Publications

Linda Kahl, Jennifer Molloy, Nicola Patron, Colette Matthewman, Jim Haseloff, David Grewal, Richard Johnson & Drew Endy (2018). **Opening options for material transfer**. *Nature Biotechnology 36:923–927.*

Rowden, SJL et al. (2018) ***Biophotovoltaics: design and study of bioelectrochemical systems for biotechnological applications and metabolic investigation*** in Photosynthesis: Methods and Protocols ed. Sarah Covshoff pp335-346. Humana Press, NY. ISBN 978-1-4939-7785-7

Vazquez-Vilar M, Orzaez D, and Patron, N (2018). **DNA Assembly Standards: Setting the Low-Level Programing Code for Plant Biotechnology.** *Plant Science 273, pp 33-41*

Pollak B, Cerda A, Delmans M, Álamos S, Moyano T, West A, Gutiérrez RA, N, Federici F & Haseloff J (2018). **Loop Assembly: a simple and open system for recursive fabrication of**

DNA circuits. *bioRxiv 247593; doi: https://doi.org/10.1101/247593* & New Phytologist, accepted for publication.

Invited Presentations

University of Edinburgh, April 2018 (Howe), University of Liverpool, April 2018 (Howe)
Integrative Cell Models for Disease Intervention workshop, Banff, June 2018 (Martins)
SB7.0: The Seventh International Meeting on Synthetic Biology, Singapore, June 2017 (Patron; Haseloff)

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 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

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
Baulcombe and Smith labs have been participating in the construction of a MoClo kit, consisting in a set of Golden Gate-domesticated DNA parts, for the green alga Chlamydomonas reinhardtii. This project is in collaboration with several European labs, led by the Lemaire group in IBPC in Paris, the Smith lab in Cambridge, and the Schroda lab in Kaiserslautern. The intention is to facilitate synthetic biology approaches in the green alga, and a manuscript describing the kit has just been published in ACS Synthetic Biology (Crozet et al., 2018). The kit itself is being made available through Addgene.

C1-2: PDRA Francisco Navarro was based in the Baulcombe lab until his departure in March 2018 to take up a position as a Senior Scientist at Axitan Ltd a London-based company

producing veterinary biologics from microalgae. During his time with OpenPlant, Francisco focused on milestones C1 and C2 in algal systems.

The 2015 and 2016 annual reports described the progress on the deliverables for milestone C1. In summary, RNA silencing modules based on miRNA-precursor sequences from the green alga *Chlamydomonas reinhardtii* were generated and optimized. A modular strategy for fast and reliable cloning was implemented, based on Golden Gate. A series of reporter systems were constructed in the alga to demonstrate that modules were able to silence gene expression in vivo. These reporter systems allowed us to characterize the effective silencing systems, targeting rules and mode of action of artificial miRNAs in *Chlamydomonas*. Strong silencing of transgenes in *Chlamydomonas* made us test several strategies to optimize gene expression. In addition, the limited availability of molecular sequences useful for gene expression in *Chlamydomonas* implied the construction of more than 500 plasmids over the last two years. The results and methodology developed here can be easily extrapolated to land plants.

The 2017 annual report describes the progress on deliverable C2. In summary, we used resources and knowledge obtained from previous deliverables to construct a simplified version of an incoherent feed-forward loop, which is the most common regulatory motif in natural gene networks. We have characterized the capacity of this circuit to buffer transcriptional noise. We expect that the results obtained with our reporter systems will help to better understand the features of the miRNA pathway in the alga.

C3: PDRA Gonzalo Mendoza-Ochoa was recruited to the Smith lab at the University of Cambridge to work on milestones C3 and C4. To know whether riboswitches of the green alga Chlamydomonas, which work through alternative splicing (as all eukaryotic riboswitches), can be transferred to other model organisms, we first asked whether introns from Chlamydomonas can be spliced by the simplest eukaryotic model organism: *Saccharomyces cerevisiae*. Based on preliminary results, 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, at least not until we have a much better idea of structure-function of the riboswitches. This is consistent with data generated by PhD student Marcel Llavero who found 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.

To be able to set up a high-throughput workflow to analyse riboswitches *in vivo*, we are constructing fluorescent reporter genes to monitor changes in the splicing status upon addition of the ligand of the riboswitch (vitamin B1) to the cell culture. Using the recently developed Chlamydomonas Golden-Gate MoClo kit (Crozet et al 2018), we have constructed a series of gene fusions between Zeocin-resistance gene (Ble), mVenus fluorescent protein gene and mCherry fluorescent protein gene. The idea is that the open reading frames (ORF) of the two reporter genes will be interrupted by the riboswitch sequence

(Promoter-ORF1-riboswitch-ORF2-terminator), so that ORF1 will report on transcript expression and ORF2 will report on splicing. Although additional tests must be performed before reaching a conclusion, preliminary results show that Ble-mVenus and Ble-mCherry produce the highest fluorescent signal; whereas fusions between mVenus and mCherry lead to reduced fluorescent signal.

C4: Aleix Gorchs Rovira and Stefan Grossfurthner (Smith lab, UCam) have produced MoClo parts for introduction of sequences into the chloroplast genome of *C. reinhardtii*. These include promoters, ribosome-binding sites, terminators and homologous regions for insertion around the genome. These will facilitate engineering of the chloroplast genome to introduce several transgenes at different sites. In addition, protocols have been established to allow chloroplast transformation by electroporation, increasing the frequency of transformants.

Regulation of chloroplast gene expression via TPR/PPR proteins encoded by the nucleus has also been established by Aleix Gorchs Rovira. NAC2 is a TPR protein required for expression of psbD, a PSII subunit. Introduction into a nac2 mutant of a construct encoding NAC2 under control of a vitamin B12-repressible promoter (PMETE) restores photosynthesis, but this can be down-regulated by increasing concentrations of vitamin B12 in the medium. The binding site of NAC2 is the 5'UTR of psbD, and if this is fused to another gene, this too will be regulated by this genetic circuit. Similarly, thiamine can be used to tune down photosynthesis in strains where MRL1 (a PPR protein required for efficient translation of rbcL) is regulated by a thiamine-repressible riboswitch (THI4RS). Crosses have resulted in generation of strains containing both PMETE-NAC2 and THI4RS-MRL1, and also the endogenous 5'UTRs for psbD and rbcL have been replaced with nonresponsive elements. These strains can now be used to introduce metabolic enzyme genes into the chloroplast and have their expression regulated by vitamins.

Evidence of the quality of the research

Crozet P, Navarro F, Willmund F, Mehrshahi P, Bakowski K, Lauersen K, Pérez-Pérez M-E, Auroy P, Gorchs Rovira A, Sauret-Gueto S, Niemeyer J, Spaniol B, Theis J, Trösch R, Westrich L-D, Vavitsas K, Baier T, Hübner W, de Carpentier F, Cassarini M, Danon A, Henri J, Marchand C, de Mia M, Sarkissian K, Baulcombe DC, Peltier G, Crespo JL, Kruse O, Jensen PE, Schroda M, Smith AG, Lemaire S (2018). **Birth of a photosynthetic chassis: a MoClo toolkit enabling synthetic biology in the microalga *Chlamydomonas reinhardtii*.** ACS Synth Biol. 2018 Sep 5. doi: 10.1021/acssynbio.8b00251

Other evidence of impact

Invited presentations

Alison G. Smith was an invited speaker at the Phyconet NIBB meeting, Warwick, August 2017. Talk title: *Algal biotechnology – is the future green?*

Alison G. Smith was an invited speaker at the Phyconet NIBB meeting 'Future of Algal Biotechnology' Cambridge, February 2018. Talk title*: Thinking outside the flask - how to do something useful with algal metabolism*

Alison G. Smith was invited to be a speaker in the MSU Plant Sciences series speaker at tMSU, Michigan, USA, March 2018. Talk title*: What can vitamin metabolism do for algal biotechnology?*

Alison G. Smith was an invited speaker at the Crossing Kingdoms Synthetic Biology meeting Cambridge, April 2018. Talk title*: Plug and play – developing tuneable gene expression in microalgae using synthetic biology approaches*

Alison G. Smith was an invited speaker at the 1st European Photosynthesis congress (ePS-1) Uppsala, Sweden, June 2018. Talk title: *Developing synthetic genetic circuits in microalgae for biotechnology and photosynthesis*

Navarro FJ. *miRNA-mediated regulation of synthetic gene circuits in the green alga Chlamydomonas reinhardtii*. WISB, Warwick University. March 2018

Conferences

Navarro FJ and Baulcombe D. *miRNA-mediated regulation of synthetic gene circuits in the green alga Chlamydomonas reinhardtii.* Synthetic biology UK, Manchester 27-28 Nov 2017.

Navarro FJ and Baulcombe D. *miRNA-mediated regulation of synthetic gene circuits in the green alga Chlamydomonas reinhardtii*. IGM symposium 2017: Plant RNA biology, Lisboa 27-28 Sep 2017

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-packages (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

Partners

Collaborations to establish a toolkit for Chlamydomonas have been established between the Smith Lab (UCam) and laboratories in Paris, Copenhagen, and Bielefeld and Kaiserslautern. This has resulted in a MoClo kit for the nuclear genome, available from Addgene. Similar kits for chloroplast transformation in *Chlamydomonas* are in progress (Crozet et al., 2018).

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

A new collaboration between the Smith lab (UCam) and Patron group (EI) has been established for an ambitious project entitled “Site-directed integration of transgenes into the nuclear genome

of plants using CRISPR/Cpf1/ssDNA", funded through an OpenPlant Fund award (start date summer 2018).

Several collaborations to accelerate the application of genome engineering technologies to plant science and agricultural biotechnology have been established. These include collaborations within OpenPlant: engineering the Marchantia genome in the Haseloff lab, UCam (Workpackage A) and engineering the potato genome in the Smith Lab, JIC (Workpackage G).

In addition, several collaborations have been established with partners outside of OpenPlant: **(i)** Sebastian Schornack is collaborating with the Henderson lab, advising on TALEN design **(ii)** Harwood (JIC) and Patron (EI) were awarded funds from the BBSRC Bioinformatics and Biological Resources 2015 call to provide targeted gene knockouts in crops using RNA-guided Cas9 nuclease to the UK research community (BB/N019466/1; Sep 2016 – August 2019). **(iii)** Patron (EI) and O'Connor (JIC) were 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, Ting Yang (Geu-Flores Lab, University of Copenhagen), visited the Patron lab (2017-2018) to establish a collaboration to edit genes in the plant alkaloid biosynthesis pathway to them as production chassis for medicinal alkaloids. **(iv)** 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. **(v)** Nicola Patron (EI) and Siobhan Brady (UC Davis) have submitted two grant application to apply genome engineering tools to plant genomes to investigate plant responses to environmental Nitrogen.

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

D2: Plastid genome vectors for chloroplast transformation.
Marchantia chloroplasts: Christian Boehm (Haseloff lab) established plastid transformation in Marchantia with a first generation of plastid chloroplast genome integration vectors that were designed with homologous ends according to the published Marchantia plastid genome. However, although the chloroplast genome was one of the first sequenced plastid genomes (Ohyama *et al*., J Mol Biol. 203:281-98, 1988), it is now clear that the biological sample was derived from cell cultures of *Marchantia paleacea* not *polymorpha*). We have re-sequenced the chloroplast genome from Cambridge isolates of M. polymorpha and re-annotated and curated the validated genome sequence (see Workpackage B). A new generation of vectors has been constructed by Dr. Eftychios Frangedakis (Haseloff lab). These are compatible with the Loop Assembly technique, are correctly homologous to target sequences in the *Marchantia polymorpha* plastid and have been been tested successfully.

Chlamydomonas chloroplasts: The Smith lab (UCam) lab has generated a range of MoClo compatible parts for chloroplast transformation of Chlamydomonas reinhardtii. We are collaborating with groups in Paris, Copenhagen, and Bielefeld and Kaiserslautern in Germany to generate a Chlamy MoClo kit for the nuclear genome, which has resulted in a joint publication (Crozet et al., 2018). The kit is being made available from Addgene. In addition, Aleix Gorchs Rovira and Stefan Grossfurthner (Smith-lab, UCam) have produced several MoClo parts for introduction of sequences into the chloroplast genome of *C. reinhardtii*. These include promoters, ribosome-binding sites, terminators and homologous regions for insertion around the genome. Protocols have been established to allow chloroplast transformation by electroporation, increasing the frequency of transformants. Regulation of chloroplast gene expression via TPR/PPR proteins encoded by the nucleus has been established by Aleix Gorchs Rovira. Strains with these regulatory circuits have been used to introduce metabolic enzyme genes into the chloroplast, the expression of which can be controlled by external applications of vitamins. Standardised and domesticated parts for a Chlamydomonas chloroplast MoClo kit will form the basis of another collaboration with other groups in the community, and an anticipated release date sometime in 2019.

D3: System for facilitating homoplasmy after chloroplast transformation. Dr. Eftychis Frangedakis and Kasey Markel (Haseloff Lab) have developed more efficient methods for gene delivery into Marchantia chloroplasts. They have optimised the biolistic delivery protocols, and employed Seashell Technology gold carrier particles for high efficiency plastid transformation. Cas9 has been codon-modified for efficient use in Marchantia and the plants tolerate presence of the gene. Eftychios has used these lines to generate ftsz1 and ftsz2 mutant plant lines. These mutants possess defects in chloroplast division, and possess fewer and larger plastids per cell. In the case of ftsz2 mutant plants, there is generally one plastid per cell. Despite this, the plants are not grossly affected under laboratory conditions. These lines may be useful for plastid transformation experiments - effectively increasing the target size, and facilitating gene conversion. We are targeting a wider set of genes to determine if the chloroplast numbers per

cell can be further reduced, and testing the lines for improved organelle transformation and homoplasty.

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 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 has been compared. A manuscript is in review. Recombinant Cas9 protein has been produced and purified and we are able to successfully deliver the protein-RNA complex to plant cells of different species. However, the efficiency of targeted mutagenesis is less than can be achieved using plasmids. Plants (*Nicotiana tabacum*, *Nicotiana benthamiana,* Arabidopsis) with disrupted selection cassettes have been created to enable efficient recovery of targeted insertion events. Attempts to repair this cassette by simultaneous delivery of nucleases and the repair template have shown that insertion at the target can be detected in protoplasts. Insertion at the target was not detected using other delivery methods methods. Regeneration of plants with targeted integrations from protoplasts is now in progress. The development of Cas9 and TALE-based ligand-inducible orthogonal transcription factors is in progress.

Evidence of the quality of the research

Publications:

Crozet P, Navarro F, Willmund F, Mehrshahi P, Bakowski K, Lauersen K, Pérez-Pérez M-E, Auroy P, Gorchs Rovira A, Sauret-Gueto S, Niemeyer J, Spaniol B, Theis J, Trösch R, Westrich L-D, Vavitsas K, Baier T, Hübner W, de Carpentier F, Cassarini M, Danon A, Henri J, Marchand C, de Mia M, Sarkissian K, Baulcombe DC, Peltier G, Crespo JL, Kruse O, Jensen PE, Schroda M, Smith AG, Lemaire S (2018). **Birth of a photosynthetic chassis: a MoClo toolkit enabling synthetic biology in the microalga *Chlamydomonas reinhardtii*.** ACS Synth Biol. 2018 Sep 5. doi: 10.1021/acssynbio.8b00251

Volpi e Silva N and Patron N (2017) **CRISPR-based Tools for Plant Genome Engineering.** *Emerging Topics in Life Sciences doi: 10.1042/ETLS20170011*

Salzman AE (2017) **Controlling Relative Gene Expression Using Orthologous Regulatory Elements: Exploring Future Prospects of Tuneable Control for the Manufacture of Natural Products.** *Masters thesis.*

Other evidence of impact

Invited talk (Patron) Gatsby Plant Science Summer School, *July 2018*
Invited talk (Patron) Essex Synthetic Biology Summer School, *July 2018*
Invited talk/Session chair (Patron) International Plant Molecular Biology (IPMB 2018), Montpellier, August 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: Agreement on a standard genetic syntax for plant DNA parts (Patron et al., 2015) has provided a coordinated approach to Milestones in all foundational workpackages, as a basis for

building an automated DNA assembly process and the establishment of a central database/registry for plant parts. This underpins Workpackages A, B, C, D and E and the genetic syntax has also been adopted for work within Workpackages F, G, H, I and J. The utility and scalability of the Type IIS assembly technique across the OpenPlant community has meant that we have looked at customised solutions that have the potential for better integrated access to genome resources and automated assembly.

Building on early work, we have adopted a two-step approach to managing standardised DNA parts. These involve a customised database solution, MarpoDB, and Benchling, a free web-based solution that facilitates part sharing and management. Benchling has proved popular as it is robust and maintenance free, and it comes with IIS assembly tools. In particular, the ROC group (Researchers for OpenPlant, Cambridge) have integrated Benchling into a shared Phytobrick-based workflow.

E2: The Haseloff lab has developed MarpoDB (http://marpodb.io; Delmans et al., 2016 in Plant Cell Physiol.), an open source database that presents the Marchantia genome from an engineer's perspective, rather than a geneticist's. The database handles the Marchantia genome as a collection of parts. We think that this break from standard genome database architecture is essential for tackling the refactoring of synthetic plant genomes. Using MarpoDB, we have identified and extracted from the Marchantia genome 400 core promoter candidates. These extracted sequences have been domesticated, removing Bsa1 and Sap1 recognition sequences if necessary, and chemically synthesised. The refactored parts have been cloned into pUAP1 a specially prepared vector designed for public distribution under the OpenMTA (vector details published in Patron et al., 2015).

The Haseloff lab has also developed 3-parameter measurement techniques for quantifying gene expression in cell suspensions in such a way that extrinsic noise is minimised and a reliable estimate of the intrinsic properties of gene promoters can be made. This relies on software models for gene expression, cell growth, and the use of a co-expressed marker to reduce variation. A computational framework has been established to allow automated analysis of microplate reader data, and this has been made available on Github (https://github.com/HaseloffLab/Platypus).

E3: PDRA Bruno Martins in the Locke lab is using synthetic circuits to rewire the cyanobacterial circadian clock. He has used *S. elongatus* constructs to probe the circadian clock and its outputs at the single cell level. He has built models of the coupling of the cyanobacterial clock, that are publicly available at the Molecular Systems Biology website and are written in SBML to enable easy sharing of the code. He is now extending this work, making the clock produce different period outputs based on his models. He has also combined modelling and experiments to understand how cell size control is driven by the circadian clock and the environment in cyanobacteria. When and at what size to divide are critical decisions, requiring cells to integrate internal and external cues. Understanding what controls this is key for building robust synthetic gene circuits in cyanobacteria. Iterating between modelling and experiments, we have shown that in both constant and light-dark conditions the cyanobacterial clock produces distinctly sized

and timed subpopulations. These arise from continuous coupling of the clock to the cell cycle, which in light-dark cycles steers cell divisions away from dawn and dusk. Stochastic modelling allowed us to predict how these effects emerge from the complex interactions between the environment, clock and cell size control. This work is in revision at *PNAS*.

Mihails Delmans and Jeanet Mante (Haseloff lab) have developed software-based classification schemes for whole-organism descriptions of the dynamics of gene expression at the cellular scale in Marchantia gemmae. The work is ongoing in collaboration with Monica Dayao the lab of Dr. Tim O'Leary (Engineering, Cambridge), with the use of deep learning techniques.

E4: CellModeller is a software package that has been developed in the Haseloff Lab for modelling of plant and microbial cellular systems. It combines genetic, chemical and physical systems for multiscale modelling of cellular growth. It has unique features in the precision and flexibility of the integrated cellular modelling environment and use of GPU-based software acceleration. The early developers of the software (Rudge, Dupuy, Mackenzie, Kan, Steiner) have moved to new positions across the world, and we have constructed a hub for continued collaboration and community building around the platform. Software code, binaries and documentation for CellModeller are freely available online via a dedicated website (www.cellmodeller.org) with a Google support forum, github repository (http://haselofflab.github.io/CellModeller/) and youtube videos (https://www.youtube.com/results?search_query=CellModeller). The latest features include cell-cell adhesion and cell shape, as well as algorithms for whole colony-scale segmentation from confocal microscopy datasets of growing microbes.

Evidence of the quality of the research

Pollak B, Cerda A, Delmans A, Álamos S, Moyano T, West A, Gutiérrez RA, Patron N, Federici F, Haseloff J (2018). **Loop Assembly: a simple and open system for recursive fabrication of DNA circuits.** *Preprint on BioRxiv. DOI: https://doi.org/10.1101/247593, accepted for publication.*

Nuñez IN, Matute TF, Del Valle ID, Kan A, Choksi A, Endy D, Haseloff J, Rudge TJ, Federici F **Artificial Symmetry-Breaking for Morphogenetic Engineering Bacterial Colonies.** *ACS Synth Biol. 6(2):256-265. doi: 10.1021/acssynbio.6b00149.* (2017).

Other evidence of impact

Public access to MarpoDB website at http://marpodb.io (Now at version 4, updated Aug 2018)

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) Started March 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: The first milestone for the Hibberd laboratory is to develop artificial protein scaffolds from bacteria and assemble these in planta for metabolic engineering. We have designed, synthesized and verified parts according to the PhytoBrick standard for the GBD, SH3, PDZ domains and their cognate ligands, all of which derive from metazoans. In addition, we have produced modules for the cohesin and its dockerin from bacterial systems that use the cellulosome complex. These parts were chosen based on previous work in bacterial systems where they have been used to increased flux through metabolic pathways. Each module has been placed into *Arabidopsis thaliana*, and shown to interact via BiFC coupled with Confocal Laser Scanning Microscopy. These DNA parts follow the Phytobrick standard and are ready for public release. In the last year, we have combined these ligands and scaffolds in a combinatorial manner to test how varying their position and stoichiometry alters end-product formation *in planta*. This has been achieved using production of an exogenous pigment not normally detected and has indicated some rules concerning use of these scaffolding components that we are now testing.

F2: The second milestone is to identify DNA motifs that generate cell specific expression in leaves. Stable transgenic lines of *Arabidopsis thaliana* have been produced, which contain epitope-tagged nuclei and ribosomes driven by cell-specific promoters. Focus has been on two promoters that drive specific expression in bundle sheath cells of leaves. By combining functional testing *via* production of truncations with computational analysis, we have identified one positive regulator in *cis* that is necessary and sufficient to drive cell specific expression in leaves, and one negative regulator that represses expression in mesophyll and veinal cells. Both are ready for public release. In the last year, we have completed analysis on two elements that work in tandem and are known as duons as they impact both on gene regulation but also protein sequence. These duons pattern gene expression in C_4 plants. Whilst they do not appear to do this in leaves of *A. thaliana*, the sequences are present and so we consider them to be part of an ancient regulatory code found in plants. We have written a second manuscript associated with a single cis-element that is necessary and sufficient to drive tissue specific expression in *A. thaliana*. This has now been deposited on BioRxiv (https://doi.org/10.1101/380188). For the second element, work is ongoing so that this can also be published.

F3: We have compiled a list of transcription factors that are preferentially expressed in bundle sheath cells of *A. thaliana*. After this process, we identified three transcription factors to follow up in detail. Of these, one interacts directly with the positive regulatory DNA element identified in F2 above. Thus, these parts can be used to drive expression of genes in designated cells.

These parts are therefore good tools to drive expression in defined cell types of leaves, and are being prepared for public release. The work detailing the identification and analysis of the transcription factors has now been deposited on BioRxiv (https://doi.org/10.1101/380188).

F4: Lukas Mueller was appointed in March 2017 as the PDRA working between the Haseloff and Webb labs. Lukas is characterising synthetic promoters and reporters for analysis and manipulation of sugar metabolism and circadian oscillations in *Marchantia polymorpha*. As part of this work, he has generated a high resolution transcriptome map of day/night and clock-dependent gene expression in Marchantia plants.

F5: The genetic architecture of the circadian system in Marchantia has been analysed by PDRA Lukas Muller (Webb/Haseloff labs). The Marchantia genome lacks homologs to CCA1 and LHY and contains only one homolog to the PRR5/7/9 family in Arabidopsis. This has informed the choice of genes for synthesis of DNA parts.
Promoter regions (3kb upstream of 5'UTR) of putative circadian clock genes and putative clock output genes were identified in the Marchantia genome, domesticated and equipped with the respective cloning tags (following the common syntax) for Loop assembly. The promoters of the following genes were generated: MpCAB2, MpRVE, MpPRR, MpCCR2. In addition, the coding sequence of the luciferase PLUS enzyme was made compatible with the loop assembly system and equipped with the respective cloning tags. Functionality of the luciferase PLUS enzyme in Marchantia was confirmed in vivo with a constitutively expressed promoter.
Transgenic lines expressing luciferase driven by the CAB2 promoter were generated in order to run high-throughput circadian assays in Marchantia. Additionally PPR::LUC fusions have been used to track rhythms in Marchantia. Circadian rhythms of promoter activity were measured using photon counting cameras. High resolution observation of dynamic patterns of gene expression is complicated by the endurance of stable fluorescent protein reporters. Muller is testing a strategy based on the fusion of synthetic degrons to fluorescent proteins to cause destabilisation of the reporter according to the N-terminal rule. This is expected to shorten the half-life of fluorescent reporters. Half-lives of the GFP signals will be measured following heat shock induction, to identify the most appropriate marker for observing circadian responses. To gain insight in to the promoter regions needed to drive rhythms in Marchantia, an RNAseq has been performed which has identified the rhythmic transcriptome of Marchantia. This being analysed for conserved promoter sequences to inform synthetic promoter design.

Evidence of the quality of the research

Reyna-Llorens I, Burgess S.J., Reeves G., Singh P., Stevenson S.R., Williams B.P., Stanley S. and Hibberd J.M. (2018) **Ancient duons may underpin spatial patterning of gene expression in C_4 leaves.** *Proceedings of the National Academy of Sciences, USA. doi/10.1073/pnas.1720576115.*

Kneřová, J., Dickinson, P.J., Szecówka, M., Burgess, S.J. Mulvey, M., Bågman, A-M., Gaudinier, A., Brady, S.M. and Hibberd, J.M. (2018) **A single *cis*-element that controls cell-type specific expression in *Arabidopsis*.** *BioRxiv* doi: https://doi.org/10.1101/380188.

Burgess, S.J., Reyna-Llorens, I., Jaeger, K., Hibberd, J.M. (2017). **A transcription factor binding atlas for photosynthesis in cereals identifies a key role for coding sequence in the regulation of gene expression.** *BioRxiv pre-print doi: https://doi.org/10.1101/165787*

Reyna-Llorens I, Hibberd JM (2017). **Recruitment of pre-existing networks during the evolution of C4 photosynthesis.** *Philos Trans R Soc Lond B Biol Sci. 372(1730). pii: 20160386. doi: 10.1098/rstb.2016.0386.*

Kümpers BM, Burgess SJ, Reyna-Llorens I, Smith-Unna R, Boursnell C, Hibberd JM (2017). **Shared characteristics underpinning C4 leaf maturation derived from analysis of multiple C3 and C4 species of Flaveria.** J Exp Bot. 68(2):177-189. doi: 10.1093/jxb/erw488.

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 (2 days); Alison Smith (JIC; 2 days); Nicola Patron (3 days)

Staff Employed
Aytug Tuncel (PDRA; Smith lab at JIC) Started Jan 2015 – Ended April 2018
Henry Temple (PDRA; Dupree lab) Started Feb 2017
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 Emiliania and Prymnesium 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 is a Cambridge BBSRC DTP funded student in the PD lab who is affiliated to the OpenPlant programme and has contributed to the aims and benefitted from the interactions and training.

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.

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 PhD 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 two 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 activity for members of all the conifer groups. We therefore have a novel xylan generated 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* GUX orthologue, which shows xylan glucuronidation activity *in vitro* after using *N. benthamiana* heterologous expression and *in vivo* in *Arabidopsis* plants (Lyczakowski et al., 2017). These plants have another novel xylan structure. We will soon use this *in vitro* system to test the different Conifer XATs 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 reporting period.

The Field group 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. 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 a new family of carbohydrate-active enzymes from Euglena, which is currently being classified by our collaborator Bernard Henrissat. 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, left 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.

Two rounds of transformation of stem explants – the second using improved constructs developed as a result of initial findings - produced plants partially edited for genes encoding one or both isoforms of starch-branching enzyme (SBE) Many of the starch granules from tubers of plants with partial editing of both 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. Aytug Tuncel also developed methods to transform and then regenerate protoplasts isolated from potato leaves. In some of these protoplast lines SBE genes were completely edited. These SBE-free lines had distinct phenotypes: there were fewer tiny and knobbly granules but each granule had deep fissures across the hilum. Excitingly, some regenerated and fully edited plants apparently lack any DNA from the construct.

Analysis of starch polymers using new UPLC-SEC analysis at the Quadram Institute revealed that starch from the fully-edited lines essentially lacked the major amylopectin fraction. It consisted almost entirely of long, linear glucan polymers in the size range expected for amylose. By contrast, and despite the abnormal granule phenotypes, starch from the partially edited lines had a near-normal ratio of amylopectin to 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. Completion of metabolic analysis of tubers in the next few months will enable publication of these results.

Evidence of the quality of the research

Yu L, Lyczakowski JJ, Pereira CS, Kotake T, Yu X, Li A, Mogelsvang S, Skaf MS, Dupree P (2018). **The patterned structure of galactoglucomannan suggests it may bind to cellulose in seed mucilage.** *Plant physiology, published in preview 5 Sept*.

Lyczakowski JJ, Wicher KB, Terrett OM, Faria-Blanc N, Yu X, Brown D, Krogh KBRM, Dupree P, Busse-Wicher M (2017). **Removal of glucuronic acid from xylan is a strategy to improve the conversion of plant biomass to sugars for bioenergy**. *Biotechnol Biofuels. 19;10:224.*

Capitalising on the infrastructure supported by OpenPlant, the Field group have made substantial headway in the discovery of new algal and bacterial carbohydrate-active enzymes with which to generate or manipulate plant glucans:

Kuhaudomlarp S, Patron NJ, Henrissat B, Rejzek M, Saalbach G, Field RA (2018). **Identification of Euglena gracilis b-1,3-glucan phosphorylase and establishment of a new glycosyl hydrolase family GH 149**. *J. Biol. Chem*. 293, 2865-2876.

O'Neill EC, Kuhaudomlarp S, Rejzek M, Fangel JU, Alagesan K, Kolarich D, Willats WGT, Field RA (2017). **Exploring the glycans of *Euglena gracilis***. *Biology 6*, 45 doi:10.3390/biology6040045.

Rejzek M, Hill L, Hems ES, Kuhaudomlarp S, Wagstaff BA, Field RA (2017). **Sugar nucleotide profiling.** *Methods in Enzymology – Chemical glycobiology* 597, 209-239.

Other evidence of impact

The above-mentioned analysis of the Euglena transcriptome has been released in the CAZy database (http://www.cazy.org/), with primary data in the process of being deposited through EBI.

Workpackage H: Tools for Engineering Plant Natural Products

Relationship to other projects/themes

The HyperTrans plant expression system (Workpackage J) is being heavily used by the Martin and Osbourn labs. This platform supports the testing and investigation of metabolic pathways and the creation of new compounds. In turn, these projects inform and enable further optimisation of this powerful tool.

Investigators

Cathie Martin (5 days); Anne Osbourn (8 days); Sarah O'Connor (4 days)
Paul O'Maille (0 days) – Left project after moving to a new position

Staff Employed

Previous:

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

Current:

Michael Stephenson (PDRA; Osbourn lab at JIC). Started February 2015
Benjamin Lichman (PDRA; O'Connor lab at JIC). Started February 2016
Ingo Appelhagen (PDRA; Martin lab at JIC). Started January 2018
Zhenhua Liu (PDRA; Osbourn lab at JIC). Started November 2017

Partners

Croda (BBSRC High Value Chemicals from Plants NIBB Proof of Concept project)
GSK (BBSRC High Value Chemicals from Plants NIBB Proof of Concept project)
Marnix Medema (University of Wageningen)
Dr. DaeKyun Ro, Associate Professor, Department of Biological Sciences, University of Calgary, Canada
Norfolk Plant Sciences has a patent granted in the USA on use of AtMYB12 to modulate metabolism.
An OpenPlant Fund grant has established new collaborations between the Osbourn and Haseloff labs for producing triterpenes in Marchantia.
Collaboration with Susan Duncan, EI, as part of OpenPlant Fund project 'Advancing the ability to image single RNA molecules at the cellular level'

Aims

Plants produce a rich and diverse array of natural products. These compounds have important ecological functions, providing protection against pests, diseases, ultraviolet 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: Projects in the Osbourn, Martin and O'Connor labs continue to identify and functionally characterise a range of DNA parts for natural product synthesis. The development of a deeper understanding of regulation of biosynthesis pathways, bioinformatics-based methods for mining plant genomes for biosynthetic gene clusters, novel practical tools and improved synthetic biology pipelines are rapidly accelerating the discovery, characterisation and practical utilization of new pathways and chemistries (e.g. see Osbourn lab publications). Several collaborative projects with industry are advancing this research in a commercial context. For example, Anne Osbourn has recently secured a BBSRC Super Follow-on Fund grant to identify genes for the synthesis of the triterpene glycoside QS-21 from soapbark (*Quillaja saponaria*) and reconstitute the pathway in *N. benthamiana* using transient plant expression technology. QS-21 is an adjuvant used in anti-malaria and shingles vaccines. Engineering the QS-21 pathway in *N. benthamiana* will serve as a flagship proof of concept project to demonstrate the power of the transient plant expression platform for accessing high-value products from plants.

OpenPlant PDRA Hans-Wilhelm Nützmann has secured a Royal Society Fellowship and has moved to the University of Bath to establish his own research group. A review article has

recently been published summarising the latest advances and understanding of Metabolic Gene Clusters in Eukaryotes (Nützman et al., 2018). OpenPlant PDRA Zhenhua Liu was recruited to the Osbourn lab to work on the biosynthetic gene clusters and contribute to this workpackage.

Understanding the evolution and diversity of biosynthetic gene clusters (BGCs) provides the basis for discovery and engineering of BGC-based metabolic pathways. PDRA Zhenhua Liu is working on systematic analysis of a large number of high-quality Brassicaceae genomes and exploring the evolution and assembly of triterpene BGCs in this plant family. Similar triterpene BGCs are widely distributed in Brassicaceae species, however, they are likely to have evolved independently in restricted taxa by recruiting the same folds of enzymatic gene families. While this is quite surprising with regards to their superficial similar structures, it reflects the remarkable adaptation of plants to fluctuating environments. This project has also employed genomic neighbourhood associations and identified certain enzymatic domains (triterpene-scaffolding genes with tailoring cytochrome P450s and acyltransferases) are significantly co-appeared at genomic loci. This will provide a toolkit which will facilitate the discovery and functional verification of numerous triterpene BGCs. The fundamental research carried out here should aid the understanding of the evolution of other classes/types of plant BGCs. This work has been written up and will be submitted for publication very soon.

The Osbourn lab has developed improved agro-infiltration methodology for production of triterpenes using the HyperTrans transient plant expression system (Reed et al., 2017). This has enabled gram-scale quantities of purified triterpene in just a few weeks, without any need for re-engineering of the host. They have also shown that this platform can be used for quick and easy combinatorial biosynthesis without the need for generation of multi-gene constructs, simply by mixing *Agrobacterium* strains harbouring different expression constructs prior to infiltration, and have used this approach to generate and purify a suite of bespoke triterpene analogs and demonstrate differences in anti-proliferative and anti-inflammatory activity in bioassays, providing proof of concept of this system for accessing and evaluating medicinally important bioactives. A video and accompanying text protocol detailing this platform has been made available to the community through publication in the Journal of Visual Experiments (Stephenson et al. 2018). PDRA Michael Stephenson, has designed a pipeline to focus efforts towards expanding our toolkit of beta-amyrin oxidising CYP450s. Systematic review of natural oleanane oxidation diversity through mining the reaxys natural project database, has afforded a database of species producing beta-amyrin compounds oxidase at orphan positions (positions at which a CYP450 is not yet known to act). This data is being used to identify those species for which sequencing data is available and which can be mined for potential new CYP450 enzymes to expand the breadth of our beta-amyrin oxidation toolkit. Work is underway to explore one particular species which is producing a beta-amyrin compound oxidised at two of the rarest positions. Differential expression analysis has revealed potential candidates closely expressed with beta-amyrin synthase enzymes. These candidates will be cloned and functionally characterised. Michael Stephenson has also produced scripts to allow the automated analysis of raw reaxys output. In addition, Michael Stephenson is working on an automated pipeline to allow the high-through-put screening of candidate enzymes though *in silico* docking analysis

against beta-amyrin to complement genomic and transcriptomic analysis methods of identifying candidate enzymes. Together with new genome mining algorithms for plant pathway discovery and advances in plant synthetic biology, this advance provides new routes to synthesize and access previously inaccessible natural products and analogs and has the potential to reinvigorate drug discovery pipelines.

PDRA Benjamin Lichman in the O'Connor group has progressed with identifying new building blocks in the biosynthesis of iridoids. Specifically, he has identified a number of biosynthetic genes that derivatize the iridoid scaffold and that modulate the stereochemistry of the iridoid ring system. An example of a gene duplication and neofunctionalization of a short chain alcohol dehydrogenase (SDR) has been discovered. The original function of the SDR was hypothesized to be to oxidize the substrate nepetalactol to nepetalactone. This homologue of this SDR has an intriguing non-redox role in controlling the stereochemical course of the ring cyclization. In sum, these new genes can now be used to generate a wide variety of iridoid analogs, which have potentially important agrichemical activity. This work is currently under revision at Nat Chem Biol. We have now developed a transformation system for Nepeta so that the metabolic profile of these valuable iridoids can be manipulated in planta. Additionally, we have sequenced additional species in the Lamiaceae and are working on the elucidation of additional iridoid scaffolds.

PDRA Noam Chayut worked on a project for plant-sourced L-Dopa production for Parkinson's treatment. L-DOPA, a product of tyrosine hydroxylation, is an intermediate metabolite in biosynthesis of violet and yellow betalain pigments, in *Beta vulgaris* (beetroot). The goal of this project was to block the turnover of L-DOPA in beetroot to allow its accumulation to levels that could enable low-tech accessible production in a plant system. Using CRISPR/Cas9-mediated genome editing, *DODA* was knocked down in hairy roots of red and in yellow beets. Hairy roots showed lower betalain pigmentation in comparison to empty vector controls in both genotypes. L-DOPA accumulation was verified by high-pressure liquid chromatography and accumulated in µg quantities. These results proved that the concept for agriculturally produced L-DOPA for pharmaceutical uses is very promising.

PDRA Ingo Appelhagen was employed in the Martin lab earlier this year. To obtain stable sources of natural colourants, he developed a novel suspension culture production system from tobacco plants constitutively expressing the MYB Rosea1 and bHLH Delila transcription factors from *Antirrhinum majus*. These cultures produce exceptionally high levels of anthocyanins and allow a robust constitutive year-around production under controlled conditions (Appelhagen et al., 2018). To expand the approach towards the production of rare natural blue colours, Ingo expressed the Rosea1 and Delila transcription factors in species that carry the genetic information to produce multiply-acylated bluish anthocyanins, such as the Butterfly Pea *Clitoria ternatea* and Brassica species. Activation of the anthocyanin biosynthetic pathway in cultures of *Arabidopsis thaliana* produced diacylated cyanidin with a blue colour at neutral pH. To obtain further training in large-scale fermentation of plant cells, Ingo recently undertook a three-month internship at Phyton Biotech in Germany, funded through a Flexible Talent Mobility Award.

Phyton Biotech runs the world's biggest bioreactor facility for plant cells and is the largest supplier of Taxane APIs.

H2: Group leader Paul O'Maille has left JIC and PDRA Don Nguyen has moved to another project. Therefore this objective is not being further continued. During his time in the O'Maille lab, PDRA Don Nguyen (O'Maille lab) identified, characterised and mutated a set of cytochrome P450 enzymes from the Asteraceae family. Enzymes characterised from *Barnadesia spinosa* have been engineered into yeast to generate oxygenated sesquiterpenes (Nguyen et al., 2016; Biochem. Biophys. Res. Commun.). Work on optimising enzymes for triterpene production is currently underway in the Osbourn lab.

H3: In alignment with work in Objective H1, the Osbourn and Martin labs have identified and characterised a number of transcription factors for control of natural product production. PDRA Hans-Wilhelm Nützmann (Osbourn lab) previously showed that plant biosynthetic gene clusters are strongly marked by Polycomb-mediated histone 3 leucine 27 trimethylation when in their 'off' state, and by the histone 2 variant H2A.Z when in their 'on' state (Nützmann and Osbourn, 2015; Yu et al, 2016), and that these features can be exploited to identify new biosynthetic gene clusters. Furthermore, he has established protocols to perform chromosome conformation capture and FISH analysis to investigate the three-dimensional positioning of biosynthetic gene clusters in the nucleus of A. thaliana. The results of these experiments suggest that metabolic gene clusters show dynamic conformational changes in their chromosome structure during active transcription and silencing.

Nützmann previously showed that an A. thaliana candidate transcription factor identified by yeast-one-hybrid assays activates promoters of metabolic gene clusters in transactivation assays. The observed activity was dependent on co-incubation with a second transcription factor. Recent data obtained by yeast-two-hybrid assays implicate a third component, a transcriptional co-activator complex, in this cluster regulatory module. Mutant analyses for all three regulatory proteins support their role in cluster regulation. The tripartite regulatory complex may represent a novel mechanism in the control of clustered metabolic pathway genes. In addition, a candidate transcription factor for a biosynthetic gene cluster from oat (the avenacin cluster) had been identified before. Functional characterisation by mutant analysis, transactivation and yeast-one-hybrid assays as well as metabolic analysis indicates that the identified transcription factor negatively regulates central cluster genes and avenacin accumulation.

PDRA Ingo Appelhagen (Martin lab) found that production of anthocyanins after ectopic expression of MYB and bHLH transcription factors is associated with limited viability of germplasm or reduced cell growth in dedifferentiated suspension cells. He isolated a truncated version of the bHLH transcription factor Delila, that was used with Rosea1 to fine-tune anthocyanin production, to increase biomass production and total anthocyanin yields.

H4: It was concluded that modifying the activity of specific transcription factors (such as MYB12) in plants was a far more effective means of engineering flux in plant production systems than

creation of synthetic metabolons (Zhang et al., 2015; Nat Comms). Consequently, emphasis has been shifted to improving our transcription factor tools and further funding was sought to improve the usefulness of AtMYB12 in engineering metabolism in tomato. Here the idea is to reduce the responsiveness of flavonoid biosynthesis to AtMYB12 by mutagenizing the promoter of Chalcone Synthase 1 which contains the AtMYB12 binding motif. This would allow ectopic expression of AtMYB12 in fruit to induce tyrosine and tryptophan biosynthesis, but to reduce the subsequent draw on these amino acid pools by flavonoid biosynthesis, without eliminating flavonoid biosynthesis completely. Mutagenesis is proposed using CRISPR/Cas9 genome editing in a collaborative project with SME Persephone Bio (funded through a proof of concept grant from the HVCfP NIBB).

H5: The Osbourn lab has tested nine promoters from the oat avenacin cluster and shown that they retain their characteristic expression patterns (in the epidermal cells of root meristems) when introduced into diverse plant species as promoter-reporter constructs. These promoters therefore represent an important resource for driving the expression of heterologous gene-sets in the root tips of plants. Three promoters from the oat avenacin cluster were used to successfully drive the expression of a three-gene pathway for a plant defence compound (dhurrin) from sorghum in *Arabidopsis thaliana* roots. These promoters have also been used successfully in a separate BBSRC-funded project to drive the expression of avenacin biosynthetic genes in wheat, the aim being to engineer wheat for production of antimicrobial defence compounds.

Evidence of the quality of research

Most recent publications:

Nützmann HW, Scazzocchio C, Osbourn A (2018). **Metabolic gene clusters in eukaryotes.** *Annu Rev Genet. 2018 Sep 5. doi: 10.1146/annurev-genet-120417-031237. [Epub ahead of print].*

Leveau A, Reed J, Qiao X, Stephenson MJ, Mugford S, Melton R, Rant J, Vickerstaff R, Langdon T, Osbourn A (2018). **Towards take-all control: A C-21β oxidase required for acylation of triterpene defence compounds in oat.** *New Phytol.* In-press Sept (2018).

Stephenson MJ, Reed J, Brouwer B, Osbourn A (2018). **Transient Expression in Nicotiana Benthamiana Leaves for Triterpene Production at a Preparative Scale.** J Vis Exp. 2018 Aug 16;(138). doi: 10.3791/58169.

Boachon B, Buell CR, Crisovan E, Dudareva N, Garcia N, Godden G, Henry L, Kamileen MO, Kates HR, Kilgore MB, Lichman BR, Mavrodiev EV, Newton L, Rodriguez-Lopez C, O'Connor SE, Soltis D, Soltis P, Vaillancourt B, Wiegert-Rininger K, Zhao D (2018). **Phylogenomic Mining of the Mints Reveals Multiple Mechanisms Contributing to the Evolution of Chemical Diversity in Lamiaceae.** Mol Plant. 2018 Aug 6;11(8):1084-1096. doi: 10.1016/j.molp.2018.06.002. Epub 2018 Jun 18.

Appelhagen I, Wulff-Vester AK, Wendell M, Hvoslef-Eide AK, Russell J, Oertel A, Martens S, Mock HP, Martin C, Matros A (2018). **Colour bio-factories: Towards scale-up production of**

anthocyanins in plant cell cultures. Metab Eng. 2018 Jul;48:218-232. doi: 10.1016/j.ymben.2018.06.004. Epub 2018 Jun 8.

Reed J, Osbourn A (2018). **Engineering terpenoid production through transient expression in Nicotiana benthamiana.** Plant Cell Rep. 2018 May 21. doi: 10.1007/s00299-018-2296-3. [Epub ahead of print] Review.

Xue Z, Tan Z, Huang A, Zhou Y, Sun J, Wang X, Thimmappa RB, Stephenson MJ, Osbourn A, Qi X (2018). **Identification of key amino acid residues determining product specificity of 2,3-oxidosqualene cyclase in Oryza species.** New Phytol. 2018 May;218(3):1076-1088. doi: 10.1111/nph.15080. Epub 2018 Mar 12.

Osbourn A (2017). **Painting with betalains.** Nat Plants. 2017 Nov;3(11):852-853. doi: 10.1038/s41477-017-0049-x.

Owen C, Patron NJ, Huang A, Osbourn A (2017). **Harnessing plant metabolic diversity.** Curr Opin Chem Biol. 2017 Oct;40:24-30. doi: 10.1016/j.cbpa.2017.04.015. Epub 2017 May 17. Review.

Sherden NH, Lichman B, Caputi L, Zhao D, Kamileen MO, Buell CR, O'Connor SE (2017). **Identification of Iridoid Synthases from *Nepeta* species: Iridoid cyclization does not determine nepetalactone stereochemistry.** *Phytochemistry 27;145:48-56.*

Reed J. et al., (2017). **A translational synthetic biology platform for rapid access to gram-scale quantities of novel drug-like molecules.** *Metabolic Engineering 42, 185-193.*

Kautsar, S. et al. (2017). **plantiSMASH: automated identification, annotation and expression analysis of plant biosynthetic gene clusters.** *Nucleic Acids Res. doi:10.1093/nar/gkx305. [Epub ahead of print].*

Fu R, Martin C, and Zhang Y (2017). **Next Generation Plant Metabolic Engineering, Inspired by an Ancient Chinese Irrigation System.** *Molecular Plant.*

Conferences (Sept 2017 - 2018 only)

Anne Osbourn has given invited keynote talks at the Natural Products and Bioactive Compounds Gordon Research Conference, Andover, US (2017), Plant Genomes & Biotechnology, Cold Spring Harbor, US (2017), Marine Natural Products Gordon Research Conference. Ventura, US (2018), Natural Products and Synthetic Biology: Parts and Pathways Keystone Meeting, Squaw Creek, US (2018), 14th International Symposium on Cytochrome P450 Biodiversity and Biotechnology, York, UK (2018), International Saclay Plant Sciences Conference 2018, Gif-sur-Yvette, France (2018).

Sarah O'Connor gave an invited talk at a keystone meeting in Synthetic Biology in Walnut Creek (January 2018)

PDRA Benjamin Lichman gave an invited lecture at ESOF2018 (July 2018) and presented at the OpenPlant forum 2018

PDRA Ingo Appelhagen has given talks at the 3rd Conference of the International Society of Plant Molecular Farming (11-13th June 2018 in Helsinki), the OpenPlant Forum (23-25 July 2018) and has given an invited lecture at the CeBiTec, Bielefeld University, Germany.

Other evidence of impact

Career development (Sept 2017 - 2018 only)

PDRA Ingo Appelhagen completed a three-month placement at Phyton Biotech,Germany, funded through a Flexible Talent Mobility Award.

PDRA Michael Stephenson (Osbourn lab) is a visiting lecturer on an undergraduate Medicinal Chemistry Course at the University of East Anglia.

PDRA Hans-Wilhelm Nützmann (Osbourn lab) was awarded a Royal Society University Fellowship and moved to the University of Bath in autumn 2017 to establish his own research group.

PDRA Benjamin Lichman (O'Connor lab) has been appointed to a Lectureship in Plant Biology at the University of York (starting November 2018) and will establish his own research group on plant secondary metabolism its biotechnological applications.

Industry partnership and Commercialisation

The John Innes Centre has filed a patent on methods of monoterpenoid production based on the work conducted by PDRA Benjamin Lichman.

PDRA Michael Stephenson (Osbourn lab) has provided valuable advice and knowledge exchange to support the establishment of the BBSRC-funded JIC spin out company Leaf Expression Systems Ltd., which was launched January 2017, and is a named investigator on a high-value chemicals from plants network business interaction voucher with a pharmaceutical company, where he is employing his natural product database mining pipeline to explore another pharmaceutically reliant scaffold.

PDRA Hans-Wilhelm Nützmann (Osbourn lab) led on a BBSRC High Value Chemicals from Plants Proof of Concept project with industry partner Croda to establish methods for chemical manipulation of plant biosynthetic pathways in cell cultures.

Public engagement and outreach

PDRA Michael Stephenson (Osbourn lab) has participated and contributed to several outreach events, including three-days of practical science workshops with the SAW trust for children at the Boomtown Festival (August 2018), and a public exhibition at the John Innes Centre open day (September 2017).

An interview with PDRA Benjamin Lichman was featured on a ‘shorts’ episode of the podcast Pythagoras’ Trousers (13th July 2018).

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); Nicola Patron (0.5 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: Robust methods have been established for co-cultivation of Marchantia spp. with Glomeromycota fungi and visualisation of the fungus colonisation (Carella et al., 2018; Carella and Schornack, 2017). In addition, a high-throughput transformation system has been developed for *Marchantia paleacea* and marker systems have been developed for secretion system pathway and tonoplast labelling.

The Schornack lab have established liverwort cultivation on vermiculite. Furthermore, they have established reproducible colonisation of several liverwort species (*Marchantia spp*., *Lunularia cruciata*) with Glomeromycota fungi (*Funnelliformis mossae*, *Rhizophagus irregularis*) in this vermiculite system and detection of the fungus using staining and high resolution confocal fluorescence microscopy. Entry of hyphae in all cases occurred through substrate oriented rhizoids. Distribution of hyphal colonisation within liverwort thalli differed markedly between *Marchantia spp*. and *Lunularia spp*. but was always restricted to the storage cell layers. Constructs have been developed for secretion system pathway and tonoplast labelling and used to confirm their functionality in *Marchantia polymorpha*.

The Schornack lab has further obtained a Marchantia transcriptome in response to filamentous pathogen colonisation and has identified a set of genes strongly upregulated during defense. From these data they could also derive a set of constitutively and highly expressed genes. Promoter sequences of these genes may be suitable for further synthetic biology approaches to achieve high level persistent expression.

This work also enabled us to identify a transcription factor (MYB14) which when expressed produces a red color. Control of this transcription factor under Heat-Shock promoter allows the generation of sectorised Marchantia thalli with different colors similar to works in angiosperms where differently colored sectors are used as reporters for cell fate and tissue development.

The Oldroyd lab employed Pierre-Marc Delaux as a postdoc, who left in Aug 2015 to take up a group leader position. The position in the Oldroyd lab has been filled in the last 6 months. Below is a summary of the progress made to date:

A *Marchantia paleacea* isolate was selected as a chassis, because of its ability to form arbuscular mycorrhizal symbiosis. A rapid transformation protocol has been developed and successfully moved onto a 96-well format, creating high throughput *M. paleacea* transformation. The genome of *M. paleacea* was sequenced using paired end libraries and illumina sequencing, providing a genome of sufficient quality for our studies in this project. Protocols for genome editing using CRISPR-Cas9 in *M. paleacea* have been established, and *NOP1* gene knockouts have been created to test the efficiency of CAS9 endonucleases in *M. paleacea*. Mutation of the *NOP1* gene creates an easily scorable and non-lethal phenotype. Unfortunately, no crispr mutants were identified from a wide screen of many different constructs, including the *NOP1* construct used in *M. polymorpha.* This suggests that we need to modify the system to get

crispr-knockouts in *M. paleacea* and our collaborator Pierre-Marc Delaux (previous postdoc on the project) is testing different promoters to drive CAS9 and the guide RNAs. We are currently using *M. polymorpha* to generate knockouts in homologs of transcription factors we are interested in. While it will not be possible to test symbiotic phenotypes in these mutants, we will be able to test some ideas about what these genes may be doing in liverworts. Once crispr knockouts are resolved in *M. paleacea* then we will generate mutants in this species also to test functions in mycorrhization.

We are currently working on a manuscript that describes the *M. paleacea* genome and comparisons to other liverworts.

Following a phylogenetic analysis, the three main symbiosis TFs (IPD3, NSP1 and NSP2) in *M. paleacea* were used in complementation assays in Medicago mutants. Expression of *MpaNSP1* in a Medicago *nsp1* mutant rescued its symbiotic defect. By contrast, *MpaNSP2* did not complement the Medicago *nsp2* mutant. To confirm this result, trans-activation of the Medicago *pENOD11* promoter, known to be targeted by these TFs, was conducted in *Nicotiana benthamiana*. Expression of *MtNSP1* and *MtNSP2* strongly activated this promoter whereas expression of *MpaNSP1* and *MpaNSP2* resulted in a weak signal. Combining *MtNSP2* with *MpaNSP1* yielded the same result than the combination of Medicago genes. By contrast, *MpaNSP2* with *MtNSP1* was only weakly active. MpaNSP1 seems fully functional in a nodulation context whereas MpaNSP2 is not.

I2: Some progress was made on this objective by PDRA Pierre-Marc Delaux (Oldroyd lab) in years 2015 - 2016 (Pub 65, 66). However, following Delaux' departure to establish his own group, and due to Giles Oldroyd's recent move from the John Innes Centre to the Sainsbury Laboratory, Cambridge University, recruitment of a post-doc to continue this objective was completed and the new person started in February 2018. The first priority has been getting CRISPR knockouts, and constructs and modules are being designed, synthesised and generated to test engineering.

Since the beginning of the OpenPlant project within the Oldroyd lab, Marchantia as a model system has been further integrated into the Bill and Melinda Gates Foundation Engineering Nitrogen Symbiosis for Africa project, which was recently awarded Phase 2 funding.

The Schornack Lab transcriptome analysis has revealed that a Marchantia syntaxin (SYP13B) whose legume ortholog is implicated in nitrogen fixing symbiosis may also play a role in intracellular colonisation by an oomycete pathogen. SYP13B is upregulated during infection and SYP13B-GFP fusions accumulate around pathogen structures inside living Marchantia cells (Carella et al. 2018, PNAS).

I3: This work will begin in the last year of the OpenPlant programme

Evidence of the quality of the research

Carella P, Gogleva A, Tomaselli M, Alfs C, Schornack S (2018). **Phytophthora palmivora establishes tissue-specific intracellular infection structures in the earliest divergent land plant lineage**. *Proceedings of the National Academy of Sciences*, 115(16), E3846-E3855.

Carella P and Schornack S (2017). **Manipulation of bryophyte hosts by pathogenic and symbiotic microbes**. *Plant and Cell Physiology*, 59(4), 656-665.

Other evidence of impact

Career development:

PDRA Pierre-Marc Delaux left OpenPlant in Aug 2015 to take up a group leader position at Laboratoire de Recherche en Sciences Végétales, Université de Toulouse, CNRS, France.

PDRA Philip Carella recently secured a prestigious 2-year postdoc fellowship from the Natural Sciences and Engineering Research Council of Canada (NSERC).

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.

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.

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.

J3: The Martin lab have developed a method for the using the CPMV-HT system for transient transformation of tomato fruits.

We have investigated the feasibility of producing protozoan proteins using the CPMV-HT system, thereby extending the range of targets this system can be used for. Specifically, we have successfully expressed a protein from *Theileria parva* (causal agent of East Coast Fever, a veterinary disease in Sub-Saharan Africa) which has been difficult to express in other systems. This opens up the possibility of novel basic research into protein structure and function, as well as serving as a potential vaccine candidate or tool for diagnostic development.

Evidence of the quality of the research

Publications

Stephenson MJ, Reed J, Brouwer B, Osbourn A (2018). **Transient Expression in Nicotiana Benthamiana Leaves for Triterpene Production at a Preparative Scale.** *J Vis Exp. 16;(138). doi: 10.3791/58169.*

Wege C, Lomonossoff GP (2018). **Virus-Derived Nanoparticles for Advanced Technologies.** *Methods and Protocols Humana press 1776 671*

Hesketh EL, Saunders K, Fisher C, Potze J, Stanley J, Lomonossoff GP, Ranson NA (2018). **The 3.3Å structure of a plant geminivirus using cryo-EM.** *Nature communications* 9 2369

Castells-Graells R, Lomonossoff GP, Saunders K (2018) **Production of Mosaic Turnip Crinkle Virus-Like Particles Derived by Coinfiltration of Wild-Type and Modified Forms of Virus Coat Protein in Plants.** *Virus-Derived Nanoparticles for Advanced Technologies Methods and Protocols* Humana Press 1776 3-17

Thuenemann EC, Lomonossoff GP (2018). **Delivering Cargo: Plant-Based Production of Bluetongue Virus Core-Like and Virus-Like Particles Containing Fluorescent Proteins.** *Virus-Derived Nanoparticles for Advanced Technologies Methods and Protocols* Humana Press 1776 319-334

Lomonossoff GP (2018). **So What Have Plant Viruses Ever Done for Virology and Molecular Biology?** *Advances in virus research* 100 145-162

Berardi A, Evans DJ, Baldelli Bombelli F, Lomonossoff GP (2017). **Stability of plant virus-based nanocarriers in gastrointestinal fluids.** *Nanoscale*

Meshcheriakova Y, Durrant A, Hesketh EL, Ranson NA, Lomonossoff GP (2017). **Combining high-resolution cryo-electron microscopy and mutagenesis to develop cowpea mosaic virus for bionanotechnology.** *Biochemical Society Transactions* 45 1263-1269

Saunders K, Lomonossoff GP (2017). **In Planta Synthesis of Designer-Length Tobacco Mosaic Virus-Based Nano-Rods That Can Be Used to Fabricate Nano-Wires.** *Front Plant Sci. 8:1335. doi: 10.3389/fpls.*

Marsian J, Fox H, Bahar MW, Kotecha A, Fry EE, Stuart DI, Macadam AJ, Rowlands DJ, Lomonossoff GP (2017). **Plant-made polio type 3 stabilized VLPs-a candidate synthetic polio vaccine.** *Nat Commun. 8, 245. doi: 10.1038/s41467-017-00090-w.*

Brillault. L., Jutras, P. V., Dashti N., Thuenemann E. C., Morgan G., Lomonossoff G.P., Landsberg M. J., Sainsbury F. (2017). **Engineering Recombinant Virus-like Nanoparticles from Plants for Cellular Delivery.** *ACS Nano. doi: 10.1021/acsnano. 6b07747.*

Conferences

In the past year, numerous talks at various meetings have been given by members of the Lomonossoff group. These include presentations at ISPMF 2018 in Helsinki, Finland, and SEB 2018 in Florence, Italy.

Other evidence of impact

Industry partnership and Commercialisation

George Lomonossoff continues to act as a consultant to the Leaf Expression Systems (LES) facility.

The HyperTrans technology is currently still being used under licence by the Canadian company Medicago for production of specific vaccines, particularly against influenza. There have been meetings between Medicago and LES in Norwich regarding progressing various collaborations. We continue to supply pEAQ vector kits containing the HyperTrans technology to laboratories worldwide under MTAs.

Public engagement, outreach and education

UK-Africa symposium The hypertrans system was introduced to 20 African researchers from 8 countries, participating in the Molecular Laboratory Training Workshop & Research Symposium organised through the JR Biotek Foundation and sponsored by OpenPlant. Participants learnt about the theory of the hypertrans system, before infiltrating Nicotiana benthamiana plants with Agrobacterium containing a GFP expression vector. GFP fluorescence was viewed four days later. (Sept 2018)

Accessible 3D Models of Molecules Roger Castells-Graells, a Ph.D. student in the Lomonossoff lab, obtained OpenPlant funding in 2016 to buy a 3-D printer to make accessible models. Briefly, this project aims to create 3D models of molecules for schools, outreach activities and scientific events. These models are used to facilitate the understanding of viral structures, polymers and synthetic biology projects. The models include structures and also pieces to be assembled as 3D puzzles and are a tool for teachers and researchers to teach about their subject in an interactive manner. In the last year, Roger has continued to use and upgrade the 3D printer to allow printing of multi-coloured models to further illustrate basic concepts and structures in biology.

The project has exceeded all the initial expectations. 3D printed models of viruses have been distributed among scientists and teachers from UK, Germany, USA, Brazil, Russia, Portugal, Spain, Jordan and Kenya. In one year, the 3D printed models produced with this project have already reached people from four continents and this will continue expanding in the following months. We are receiving requests from scientists and teachers to produce more models that include, for example, viruses, proteins, nanoparticles, self-assembly models and bacteria. Roger showcased his models at the OpenPlant Curriculum Hacks event to researchers, biomakers and teachers.

John Innes Centre Open Day (September 2017). Several members of the Lomonossoff lab ran a stand at the John Innes Centre open day engaging the public and teaching them about virus structures, how scientists can make virus-like particles in plants to be used as vaccines and for much much more. The open day attracted a crowd of around 3,000 people across the day.

Norwich Biomakers: Prof George Lomonossoff gave a talk about the research in his lab at a Norwich Biomakers event entitled “Building Nanostructures in Plants”. Roger Castells-Graells got hands on with the group of participants and enabled them to craft their own virus like particles. Norwich Biomakers is an interdisciplinary group of adults interested in learning across the disciplines and applying their skills to solve novel challenges.

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.
Alexandra Ting (Communications Officer for OpenPlant and SynBio SRI). Started January 2017

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.

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.
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-institute projects. Over the last year, we have funded 19 new projects:

December 2017 OpenPlant Fund projects:

> **A cell-free sensor platform for the quantification of arsenic concentrations in drinking water.** Genevieve Hughes (Earth Science, UCam), Elise Siouve (Biotechnology, UCam), Carolina Orozco (Biotechnology, UCam), Sina Schack (Biochemistry, UCam), Lisa Hecker (Biophysics, UCam), Alexandru Grigoroiu (Biomedical Engineering, UCam), Sammy Mahdi (Electrical Engineering, UCam, James Vereycken (Organic Chemistry, UCam), Francesco Tonolini (Physics, UCam), David-Benjamin Grys (Electrical Engineering, UCam), Tess Skyrme (Aerospace Engineering, UCam), Ralf Mouthann (Pysics, UCam)
>
> **Cell-free diagnostics for the surveillance of livestock viruses.** Laura Mitchell (Chemistry, UChem), Raghd Rostom (Wellcome trust Sanger Institue and UCam), Emily Groves (Medicine, UCam), Andre Zylstra (Babraham Institute and UCam), Punika Ratchachittapong (summer intern, UCam)
>
> **Development of Manufacturing Capability for Rare Sugar Nucleotides.** Tom Simmons (Glycoscience, UCam), Jan Lyczakowski (Biochemistry, UCam), Henry Temple (Biochemistry, UCam)
>
> **Engineering of Chlamydomonas reinhardtii to produce betalain pigments and the use of riboswitches to direct metabolic flux.** Alfonso Timoneda (Plant Sciences, UCam), Dr Payam Mehrshahi (Plant Sciences, UCam),
>
> **CGSENS: Visualization of CG methylation using a fluorescence protein biosensor.** Dr Marino Exposito-Rodriguez (Biological Sciences, UEssex), Dr Sara Lopez-Gomollon (Plant Sciences, UCam),

Design of synthetic plant and mammalian gene regulatory networks using nonparametric Bayesian approaches. Marc Jones (Computational and Systems Biology, JIC), Iulia Gherman (Biology, UYork), Anastasiya Sybirna (Wellcome/CRUK Gurdon Institute, UCam)

GardenSeq Chasing the invisible diversity of microbial life forms in vegetable garden beds with a portable DNA-sequencer. Maximilian Stammnitz (Veterinary Medicine, UCam), Meltem Gürel (Computational Biology, UCam), Philipp Braeuninger-Weimer (Electrical Engineer, UCam), Daniel Elías Martin-Herranz (Bioinformatics, Welcome Trust Genome Campus, UCam), Daniel Kunz (Wellcome Trust Sanger Institute, UCam), Christian Schwall (Sainsbury laboratory, UCam)

Plug and play synthetic biology education resource. Dr Katia Smith-Litière (Biomakespace), Dr Payam Mehrshahi (Plant Sceince, UCam), Patrick Hickland (Plant Science, UCam), Tony Naggs (Biomakespace), Marek Balint (Biomakespace), Roger Mason (Biomakespace)

Single cell pollen meiosis screening in wheat. Ashleigh Lister (Earlham Institute), Dr Iain Macaulay, (Earlham Institute), Dr Matt Clark (Earlham institute), Prof Graham Moore (Crop Genetics, JIC), Prof Peter Shaw (Cell and developmental Biology, JIC), Dr Azahara Martin (Crop Genetics, JIC), Dr Lola Santome (Crop genetics, JIC)

Harvesting the genetic value of interspecific wheat introgressions. Tobias Barber (NIAB and UCam), Dr Alison Bentley (NIAB), Dr Keith Gardner (NIAB) Dr Chris Wright (Earlham Institute), Dr Jaroslav Dolezel (Centre of Plant Structural and Functional genomics, Czech Republic)

Identifying nutrient-status dependent elements regulating the wheat transcriptional response to neighbours. Stéphanie Swarbreck (Plant Sciences, UCam), David Swarbreck (Earlham Institute)

Bench-top Controlled Environment Growth Chamber for Speed-Breeding and Crop Transformation. Oscar E. Gonzalez-Navarro (Quadrum Institute and JIC), Ricardo H. Ramirez-Gonzalez (Crop Genetics, JIC), Sreya Ghosh (Crop Genetics, JIC), Marcela Mendoza-Suarez (Plant Science, Uni. of Oxford), Luis Hernan (Architecture, Newcastle Uni.), Carolina Ramirez-Figueroa (Architecture,Newcastle Uni.)

July 2018 OpenPlant Fund projects:

Extending the type IIS toolkit for subcellular localisation in Marchantia. Connor Tansley (University of East Anglia), Susana Sauret-Gueto (University of Cambridge), Linda Silvestri (University of Cambridge)

Developing a frugal transcription factor relative affinity measurement pipeline (TRAMP). Yaomin Cai (Earlham Institute), Will Nash (Earlham Institute), Susana

Sauret-Gueto (University of Cambridge), Eftychios Frangedakis (University of Cambridge)

Site-directed integration of transgenes into the nuclear genome of plants using CRISPR/Cpf1/ssDNA. Gonzalo Mendoza-Ochoa (University of Cambridge), Nandor D Hegyi (University of Cambridge), Aleix Gorchs (University of Cambridge), Payam Mehrshahi (University of Cambridge), Oleg Raitskin (Earlham Institute), Quentin Dudley (Earlham Institute)

Harnessing cytosine DNA hypomethylation to explore the potential for crop improvement in wheat. Natasha Elina (University of Cambridge), Ian Henderson (University of Cambridge), Alison Bentley (National Institute of Agricultural Botany), Keith Edwards (University of Bristol)

Receptor-like kinases at the initiation and maintenance of AM symbiosis. Chai Hao Chiu (University of Cambridge), Héctor Montero Sommerfeld (University of Cambridge), Uta Paszkowski (University of Cambridge)

Visualising genetic circuits in space and time with paper-based cell-free translation. Zakir Tnimov (MRC Laboratory of Molecular Biology, Cambridge), Charlie Morgan (MRC Laboratory of Molecular Biology, Cambridge)

Optimising open enzyme purification using 3D-printing and automation. Jenny Molloy (University of Cambridge), Clayton Rabideau (University of Cambridge), Stefan Grossfurthner (University of Cambridge), Harry Akligoh (Kumasi Hive, Ghana), Quentin Dudley (Earlham Institute)

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 (see outputs below).

OpenPlant is encouraging the development of cell-free technologies through the OpenPlant Fund and Biomaker Challenge, as these promise low-cost options for testing, diagnostics and production of useful biocompounds. The Cambridge Synthetic Biology SRI received a nine month grant to host monthly cell-free lunches where a range of students, academics and industry researchers are able to come together to present, share, and discuss cell-free technologies. This is a good example of overlap between the two initiatives, and events are advertised to the OpenPlant community, who are engaging with these meetings. Several cell-free hands-on workshops have also been organised, most recently in collaboration with Dr

Quentin Dudley (Patron Lab, Earlham Institute) as part of an OpenPlant Fund project to established an in-house E.coli CFPS system for testing of a range of proteins from plants.

In addition to hardware development and the plant synthetic biology and cell-free biological projects the last year has seen two projects working with African researchers: 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. A second project worked with the Kumasi Hive and Lab_13 project in Ghana to develop resources for teaching synthetic biology in schools in a low-resource setting.

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.

There has been a huge amount of interest in the Biomaker Challenge. In two years, we have funded 61 projects involving close to 200 participants from Cambridge, Norwich and beyond. Projects have included everything from rodent physiology monitoring beds for pre-clinical experiments to growth chambers for the sustainable production of mushrooms, and some teams have gone on to publish papers or receive additional funding from external sources to expand on the projects initiated through this challenge.

In our second year of the challenge, we implemented a 5-session training series aimed at teaching basic hardware and software for scientists. Using the visual programming interface XOD, along with hardware available in the starter kits, we tackled some of the bottlenecks and difficulties that non-programmers face when building electronics. Overviews and tutorials of each session were published on our website (www.biomaker.org). We hope to expand and improve upon this training series in the coming years.

Outputs from the OpenPlant Fund and Biomaker Challenge projects are now 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.

In September 2017 the Norwich Biomakers group was established by Nicola Patron (EI), Aaron Hunter (Hethel Innovation and GoBio) and Colette Matthewman. Norwich Biomakers brings together an interdisciplinary network interested in the cross-over of biology with design,

technology, engineering, electronics, software, art and much more, and has gained a membership of 180 members in the first year. The network has been developed for members to learn from each other about the latest technologies and science advances, share ideas and skills and shape project. We have run ten sessions over the first year, varying from lab practicals on building plant-microbial fuel cells with Dr Paolo Bombelli from the University of Cambridge, to beginner workshops in building electronic circuits with Arduinos and in 3D design and printing, as well as having themed sessions on speed breeding technologies, electricity generated from bacteria, and cyanobacteria as biofactories. The network has led to several new collaborations feeding into OpenPlant Fund and Biomaker Challenge projects, as well as a strongly interdisciplinary proposal submitted to the plant-powered camera trap challenge (an OpenPlant Fund sponsored project).

Jenny Molloy has secured a Shuttleworth Foundation Research Fellowship and is studying the role and impact of open approaches to intellectual property for a sustainable and equitable bioeconomy, while remaining at the University of Cambridge. Jenny's work aligns well with the aims of OpenPlant and we continue to enjoy a productive collaboration.

Evidence of the quality of the research

K1: Project outputs from the OpenPlant Fund are represented on www.biomaker.org and have been very diverse, from the creation of DNA parts, to instrumentation, software, workshops and policy reports. Some of the projects have received a second round of funding to further develop their tools. Project outputs include a growing number of publications, documented hardware and software and policy reports.

K2: Projects from the Biomaker Challenge have also been diverse and have been documented on github and hackster.io where a growing community of biomakers are submitting detailed write-ups of their projects under the Biomaker group that was created specifically for the Biomaker Challenge (https://www.hackster.io/biomaker/). All projects are also captured on www.biomaker.org with links to the relevant documentation.

Publications:

Yu, Z., Boehm, C.R., Hibberd, J.M., Abell, C., Haseloff, J., Burgess, S.J., Reyna-Llorens, I., (2018). **Droplet-based microfluidic analysis and screening of single plant cells.** *PLoS One 3;13(5):e0196810. doi: 10.1371/journal.pone.0196810.*

Scott D and Berry D (2018). **Genetic Resources in the age of the Nagoya Protocol and gen/genome synthesis.** http://www.stis.ed.ac.uk/__data/assets/pdf_file/0005/247595/Nagoya_workshop_report_web.pdf

Nuñez I, Matute T, Herrera R, Keymer J, Marzullo T, Rudge T, Federici F (2017). **Low cost and open source multi-fluorescence imaging system for teaching and research in biology and bioengineering.** *PLOS One* https://doi.org/10.1371/journal.pone.0187163

Juhas M, Ajioka JW, (2017). **T7 RNA polymerase-driven inducible cell lysis for DNA transfer from Escherichia coli to Bacillus subtilis.** *Microb Biotechnol.; 10(6):1797-1808. doi: 10.1111/1751-7915.12843.*

Sotta, N., Duncan, S., et al. (2017). **Rapid transporter regulation prevents substrate flow traffic jams in boron transport.** *eLife; 6:e27038. DOI: 10.7554/eLife.27038*

Other evidence of impact
Nuñez and colleagues won the PLOS OpenSource Toolkit Channel prize for their PLOS One article on a low cost & open source multi-fluorescence imaging system, listed above (Nuñez et al., 2017)

As part of an OpenPlant Fund project, Tobias Wenzel (University of Cambridge) established an open source hardware documentation software and online repository called DocuBricks (www.DocuBricks.com) for modular and complete documentation of hardware projects. Thirteen projects have been documented on this website to date. Feedback from users demonstrates that the tool is easy to use and helpful in a wide range of hardware projects and saves documentations in a modular and accessible XML format. Tobias has since gone on to found the Journal of Open Hardware.

The workshop leading to the report “Genetic Resources in the age of the Nagoya Protocol and gene/genome synthesis” was attended by Molly Bond from BrisSynBio and a blog was posted on the BrisSynBio ethics blog drawing attention to the published report, evidencing one of many links between the UK SynBio Research Centres.

Dr. Jennifer Deegan established a website to document images taken using her focus stacking photography system that she has established using funds from the Biomaker Challenge and subsequently the OpenPlant Fund, using it to take clear and deep focused photographs of tiny plant specimens: http://chlorophyllosophyimages.blogspot.com/. Jennifer has used this setup to photograph the scientific specimens of her collaborators, who could not get the images they need through standard photography or microscopy. Jennifer has developed a series of instructional videos explaining how to build and use the setup. These are hosted on YouTube.

News article from John Innes Centre website about Susan Duncan’s work, made possible through an OpenPlant Fund grant:

We have also instigated a regular Biomaker Blog Feature in the monthly OpenPlant Newsletter. Blogs can be found here: https://www.openplant.org/news/

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
Alexandra Ting (OpenPlant and SynBioSRI Communications Officer). Started January 2017
Samantha Stebbings (Administrator). Started November 2017

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
Milestones for Workpackage L are annual and will have been completed for the third year by the end of August, with the organisation of the fourth OpenPlant Forum (L1.1), the establishment of a new working group on new models for documentation, distribution and publication by bioengineers (L1.2), and the completion of a wide-ranging set of activities with the SAW Trust (L2).

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 2018 OpenPlant Forum focussed on engineering plants for bioproduction, exploring a range of examples of products that can be made in plants, tools for engineering for bioproduction and both the global challenges and opportunities for harnessing biodiversity. The Forum was coupled with the OpenPlant Fund pitches, and a Curriculum Hacks event showcasing educational activities and resources developed through OpenPlant Fund and Biomaker Challenge projects, as well as by partners such as Science and Plants for Schools (SAPS), Cambridge Biomakespace, and the University of East Anglia.

L1.2: There have been four OpenPlant working groups to date:

1. **Intellectual Property and Innovation**. In 2015, the first working group was established to consider IP solutions for bioengineering, with a focus on the OpenMTA. The OpenMTA now has a web presence hosted on the BioBricks website at https://biobricks.org/openmta/, including a video description of the aims of the OpenMTA and a set of short video case studies. These videos have involved a number of OpenPlant researchers in the making. A report from IP working group report has been published on the OpenPlant website and printed copies have been widely distributed. A commentary paper “Expanding options for material transfer via the OpenMTA” has been accepted for publication in Nature Biotech and a collection of signatories to the OpenMTA Master Agreement is being accrued. This includes the OpenPlant institutes of JIC, EI and the University of Cambridge. Most importantly, Addgene are working to implement the OpenMTA as an optional transfer agreement for use with their plasmid distribution service so that in the future researchers will be able to choose which transfer agreement they deposit materials under (currently all materials are deposited under the restrictive UBI MTA).

2. **Nagoya Protocol and Synthetic Biology**. In 2016, and OpenPlant Fund project, led by social scientists at the University of Edinburgh, gathered a working group to discuss

"Genetic resources in the age of the Nagoya Protocol and gene/genome synthesis". This working group included a range of experts in the field, from practicing synthetic biologists to social scientists, data infrastructure experts, regulators and biological collection managers from the London Natural History Museum and Kew Gardens. A report has been published in 2018 through the OpenPlant (https://www.openplant.org/reports/) and University of Edinburgh websites, and printed copies have been distributed at the OpenPlant Forum and other relevant events.

3. **Open Curriculum Development**. The 2017 working group focused on curriculum development and teaching resources for fast and frugal biotechnology. The first meeting of the working group took place in July 2017, immediately after the 2017 OpenPlant Forum. A range of key players were invited, including researchers at the cutting edge of the technology, educationalists and outreach experts. The working group focused on the educational opportunities offered by cell-free systems and freeze-dried components to provide a platform for teaching synthetic biology at low-cost and without use of GMOs. Work continues on the development of cell-free technologies both for research and for education through a number of OpenPlant Fund projects, and monthly lunchtime sessions focused on cell-free systems are hosted at the University of Cambridge to bring together students, academics and industry researchers to present, share, and discuss cell-free technologies (https://www.synbio.cam.ac.uk/initiatives/cell-free-synthetic-biology). OpenPlant researchers led by Jim Ajioka have been successful in raising a £1.8M EPSRC-GCRF grant towards low-cost virus diagnostics with partners in Africa (Feb 2018).

4. **New Models for Publication**. In 2018, a new working group was established to investigate new models for documentation, distribution and publication by bioengineers - in particular focussing on documentation and distribution of characterisation data for DNA parts. The working group convened at a workshop on 26 July 2018, following the OpenPlant Forum and involved a number of key players including Dr Linda Kahl (BioBricks Foundation), Dr Joanne Kamens (Addgene), Dr Richard Sever (BioRxiv at the Cold Spring Harbour Laboratory), Prof Claudia Vickers (Leader of CSIRO Future Science Platform in Synthetic Biology), and Steve Evans (Dow AgroSciences). The workshop addressed the question whether with the increased use of standardised, reusable DNA parts and circuits there is a need for better ways of sharing characterisation data. It included technical discussion about new models for bio-technical publication based on user-generated content, including web-based platforms and ways of working that have emerged from the electronics and software industries.

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 seven OpenPlant-themed workshops in primary schools, designed and delivered by research scientists in collaboration with SAW, influencing the educational approach to these topics. We have engaged with the public by delivering inspiring interactive exhibits at the Norwich Science Festival ("The Power of Plants", Oct 2017) and Cambridge Science Festival ("Synthetic Biology for the Senses", Mar

2018). We also delivered science activities to engage children in the kids areas at Latitude Festival (2016) and in Kidztown at the Boomtown Fair music festival with stands entitled “Marvellous Medicines” and “The Mad Hatters Tea Party” (Aug 2017 & 2018 respectively). Latitude pulls in around 35,000 visitors while Boomtown attracts over 50,000 visitors annually.

SAW developed a training workshop to enable dissemination and to share best practice with other research centres. Jenni Rant delivered two of these workshops for SynthSys and the UK Centre for Mammalian Synthetic Biology (University of Edinburgh), and a similar collaboration has been established with Warwick Integrative Synthetic Biology Centre, to deliver workshops in the coming year. In 2018, an International conference established by the SAW Trust in collaboration with Norfolk County Council brought together education specialists from across the world locally and internationally to share learning platforms and develop ideas, in which SAW presented its school projects with OpenPlant.

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. The first pilot workshop ran in June 2017. Two additional workshops are to be held in October 2018 during the Norwich Science Festival, one with the public and a second with a group of researchers, enabling them to reflect on their own research. The workshop is now featured on the OpenPlant website https://www.openplant.org/global-garden and the pilot workshop is being written up for a publication.
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. DNA Dave has since made appearances at Cambridge Science Festivals (2017 & 2018) and Norwich Science Festival (2017). Adults and children alike have found this tool both informative, accessible and fun, and teachers claimed that they saw “lightbulb” moments when their pupils understood these complex scientific processes that were proving difficult for them to grasp in a classroom environment. Since her employment, Samantha Stebbings has been exploring opportunities for the use of DNA Dave in schools to complement curriculum learning. We now have a DNA Dave design blueprint which was produced by a local engineering centre and will be made available for future projects. DNA Dave will also be used in pilot science lessons later this year at local high schools.

OpenPlant - SAW Primary School Workshops

3. In 2018 a SAW project was developed and run by Samantha Stebbings on the theme of Biodiversity. Read more about it in this blog.

4. In spring 2017, two SAW Trust primary school workshops were run on the theme of plants as green factories for producing vaccines and high value natural products. The latter was filmed to show SAW in action: http://sawtrust.org/news/saw-in-action/
5. Over the summer of 2017, two primary school workshops were organised by OpenPlant intern, Emma McKechnie-Welsh, under guidance of Jenni Rant, covering biodiversity, plant evolution and genetics. Read more about it in this blog.
6. In 2016, OpenPlant Fund grant award winners, Carlos Lugo and Marielle Vigouroux, worked with The SAW Trust to develop and deliver a 1-day workshop for Year 6 pupils at Stapleford Community Primary School (South Cambridgeshire). More details can be found in this blog on the OpenPlant website.
7. In 2015 the first OpenPlant-SAW workshop was run at Ludham primary school. A full day workshop with 32 kids (age 8-11 year-old), covering DNA, mendelian genetics, synthetic biology and Marchantia as a model plant. Read about it in this blog post.

OpenPlant at Science and Music Festivals

8. OpenPlant and the SAW Trust have delivered fun and engaging science activities at Latitude mixer arts festival (2016) and Boomtown Festival (2017 & 2018). This year we delivered a 'Mad Hatters Tea Party' in Kidztown at the Boomtown Festival. The music festival attracted 60,000 people this year. Our interactive workshop invited children to join us for a science inspired tea party where they got to make their own tasty treats, tie dye tissues and secret messages using invisible ink. Throughout the tea-party, children learned about the different uses of plant products. They made their own edible jelly balls using alginate extracted from seaweed, made rapid ice-cream using different plant flavours, fizzy sherbet and invisible sodium bicarbonate ink which they revealed with red grape juice. They also extracted plant pigments for activities inspired by all the bright colours in Wonderland, including berry tie dye tissues and colour changing flowers and celery. Read more about it in this blog post.
9. OpenPlant, the SAW Trust and Cambridge SynBio SRI have teamed up for the past three years (2016, 2017 & 2018) to deliver a range of exciting interactive science at Cambridge Science Festival with a focus on Synthetic Biology. In 2018 we braved sub-zero temperatures to explore plant natural products with those who dared to venture out in the chilly weather. Visitors could extract their own plant pigments, learn about synthetic biology and how these pigments can be made in plant cell cultures and extracted for use as colorants in food. In addition, DNA Dave the robot came along to explain how proteins are made using the instructions in DNA. He continues to be a huge hit with public of all ages, and we were really pleased to see how adults, teenagers and young children alike enjoyed learning with DNA Dave. Read more about it here.
10. The first Norwich Science Festival took place in 2016, and OpenPlant were there with a weekend-long interactive exhibit showcasing a range of products that can be made in plants. The exhibit incorporated OpenPlant Fund project "Accessible 3D models of molecules", including a public effort to make the largest virus like particle possible out of paper "protein pieces", and researchers from the University of Cambridge introducing microalgae to the public. Read about it in this blog post. In 2017, we ran a one-day

exhibition where participants could learn about the various uses for plant natural products and could extract colour-changing pigments from plants and learn about the chemistry of these colour changes. We produced a handout so that visitors could read more about the science and try out some experiments at home. The exhibit was so popular that we had a continuous queue for our activities all day long. We estimate that we interacted with over 400 people across the course of the day.

Evidence of the quality of the research

Publications

Kahl L, Molloy J, Patron N, Matthewman C, Haseloff J, Grewal D, Johnson R & Endy D. **Expanding options for material transfer via the OpenMTA.** *In Press. Manuscript accepted to Nature Biotech.*

Scott D and Berry D (2018). **Genetic resources in the age of the Nagoya Protocol and gene/genome synthesis.** *Workshop report.*

Molloy J and Kahl L (2016).**Towards an Open Material Transfer Agreement.** *OpenPlant Intellectual Property Working Group Meeting Report.*

Other evidence of impact

Series of videos and case studies on the OpenMTA website: www.openmta.org

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
Alexandra Ting (OpenPlant and SynBioSRI Communications Officer). Started January 2017
Samantha Stebbings (Administrator). Started November 2017

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: This year Jenny Molloy left OpenPlant to take up an independent Shuttleworth Fellowship, and is no longer involved in the coordination of OpenPlant activities. Colette Matthewman has taken on extra responsibility for cross cutting activities, such as the OpenPlant Fund. Alexandra Ting (OpenPlant and SynBioSRI Communications Officer, Cambridge) has taken on some of the Cambridge OpenPlant coordinator duties, and works closely with Colette to ensure the coordination of activities between the Cambridge and Norwich sites continues to work effectively and to support cross-cutting activities. Communication between the coordination group is regular, including email exchanges, Skype calls and visits between the two sites. The coordination group uses Basecamp for project management and to store and share final documents, while Google Drive is used as a regular file share system.

M2: We continue to hold quarterly management group meetings. These meetings are held in person as far as possible. During these meetings we review the progress of the programme and discuss strategies and future planning. The minutes from the management meetings are shared with the Science Advisory board via Basecamp.

M3: The fourth Science Advisory Board Meeting took place on 25 July 2018 following the annual OpenPlant Forum. The response of the SAB to OpenPlant research reported at the Forum and other activities discussed in the meeting was highly positive. This year discussions focussed mainly on the outcomes of the midterm review, opportunities for the future and the impact of OpenPlant. Tim Fell has stepped down from the advisory board due to other time pressures. Marcus Gerschater (also from Synthace) has agreed to join the SAB in his place. The SAB are happy with this new appointment.

M4: OpenPlant researchers came together twice this year, for the midterm review meeting in February and the OpenPlant Forum in July. In addition, sub groups have come together at more focussed meetings. The ROC group in Cambridge continues to enable interaction and exchange between post-docs and PhD students. Also, the OpenPlant Fund and Biomaker Challenge mixer events provide great opportunities for ideas exchange between researchers in

Norwich and Cambridge. We continue to share information about a variety of activities and opportunities with OpenPlant researchers and the wider community via email and through the OpenPlant Newsletter and twitter.

M5: In light of Jenny Molloy's departure, Colette has taken on responsibility for managing the OpenPlant Fund, organising mixer events in advance of the submission deadlines, assisting applicants with the identification of potential collaborators and arranging a public pitching event. For each call a panel of judges, made up of OpenPlant group leaders and external industry partners, is convened to assess the applications and pitches against the fund guidelines. Management of the Biomaker Challenge is being led by Alexandra Ting and Jim Haseloff in Cambridge, and coordinated with Colette for engagement of the Norwich community. This year, management of the Biomaker Challenge included the organisation and implementation of a series of workshops to introduce scientists to programming and electronics (https://www.biomaker.org/training-session-one/). These workshops were well attended and well received, including participation from Norwich researchers.

M6: The OpenPlant Forum took place at the John Innes Centre from 23-25 July 2018 with over 130 attendees registered for the three days of events. Participants included representatives of twelve companies (incl. start-ups, SMEs and multinationals) and three charitable foundations. Several attendees from Edinburgh SBRC also joined us for the meeting.

The next OpenPlant Forum will be held at Murray Edwards College in Cambridge from 29-31 July 2019.

M7: Samantha Stebbings has been employed as OpenPlant administrator, and works in addition to general admin, works with Colette and Jenni Rant to coordinate OpenPlant engagement activities, especially with the SAW Trust. This year has seen a busy schedule of activities in schools, at science festivals, at music festivals, and on the John Innes Centre site. In addition to the SAW Trust activities, a very successful PIPS internship was completed by second year PhD student, Camilla Stanton, with Colette, to research educational resources, design a template for recording activities as teaching resources and to test and deliver write-ups of three synthetic biology teaching resources for the SynBio for Schools OpenPlant Fund project.

M8: OpenLabTools activities are now delivered through the Biomaker Challenge. In addition, this year OpenPlant supported the Makerere University iGEM team in Uganda through the Biomaker Challenge, and provided Biomaker starter kits to Bahir Dar University, Ethiopia, Mansoura University, Egypt and Pretoria University, South Africa, with a view to full inclusion in 2019.

Evidence of the quality of the research

An OpenPlant Handbook was distributed at the midterm review, highlighting the progress and impact that the programme has made over the last three years, through its research programme, cross-cutting complementary activities, outreach and through the OpenPlant Fund.

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 chaired by Tom Knight.

TRAINING AND CAREER DEVELOPMENT

OpenPlant has recruited a community of highly motivated and proactive PhD students and PDRAs who benefit from opportunities at the University of Cambridge, John Innes Centre and Earlham Institute as well as resources for collaboration and training made available directly through OpenPlant.

OpenPlant PDRAs have access to a range of excellent training and career development opportunities at their host institutes and have funding available to attend external workshops critical to their projects and personal development. To broaden the opportunities available, Colette and Alexandra have been working with organisers of training and career activities in their respective institutes to ensure that events relevant to OpenPlant researchers are accessible to participants from both sites. This has been very successful, with numerous courses being opened up to wider participation from OpenPlant researchers. A Slack account established for online communication between the wider OpenPlant community has proven to be a good tool for sharing information about training opportunities across the sites.

To complement existing training and career development opportunities at the OpenPlant institutes, OpenPlant have funded and organised numerous specialised workshops, detailed above.

The OpenPlant Fund and Biomaker Challenge play an important role in enabling researchers, with the support of their group leaders, to independently apply and obtain funding for collaborative interdisciplinary projects of interest that they might not otherwise be able to pursue. Applicants gain experience in starting up and shaping collaborations, proposal writing and pitching through the application process.

Training of undergraduate students continues to play a key role in OpenPlant's aim of supporting development of open tools and as a pathway to interdisciplinary exchange. The new Biomaker Challenge is open to undergraduates (as well as postgraduates, postdoctoral workers and faculty), and OpenPlant has developed a collaborative relationship with the Cambridge University Synthetic Biology Society and the Cambridge Biomakespace, and is supporting undergraduate activities in this way.

ADDED VALUE

The OpenPlant initiative is providing support for Open Technology Week in Oct 2018 in Cambridge. The week will include activities for developing fundamental tools for synthetic biology and beyond, including a Biomaker Fayre - as part of the Biomaker Challenge and Open Technology Forum. These activities will focus on access, openness and enabling technologies and attract a strongly interdisciplinary group of participants.

OpenPlant continues to work closely with The Science Art Writing Trust (SAW) to take a different approach towards stimulating discussion and co-learning on themes relevant to the use of synthetic biology to address grand challenges with different groups of participants. Although the approach has been used mainly in schools so far, we have demonstrated that SAW is a highly effective vehicle for engaging people of all ages in discussions about science. It uses science as a meeting place for exploration, and draws on visually exciting scientific images and cross-disciplinary approaches involving art and poetry to demystify synthetic biology and capture the imagination of groups from primary school to adulthood. This approach gives people the space to explore the concepts, potentials and ethics of synthetic biology through various media that are accessible to people who are less engaged with science. SAW activities are outlined in Workpackage L description.

IMPACT

Synthetic Biology offers the prospect of reprogrammed biological systems for improved and sustainable bioproduction. While early efforts in the field have been directed at microbes, the engineering of plant systems offers the even greater potential benefits of complex metabolism, huge scale and low costs. The OpenPlant initiative aims to (1) promote innovation by accelerating the development and exchange of underpinning tools and techniques in plant synthetic biology, and to (2) facilitate outreach, policy discussion and international development.

The first four years have seen substantial progress in promoting a two-tier approach to managing intellectual property, as we explore ways of implementing less restrictive models for distributing low-level tools and components for plant biotechnology. Innovation in a young field like Synthetic Biology requires freedom to operate. We believe that our steps to facilitate free exchange of DNA parts and tools will substantially speed the take-up of new technologies in plant synthetic biology, and foster innovation and entrepreneurship in the UK and beyond.

The introduction of standards for the assembly of characterised DNA sequences and the establishment of Registries for standardised parts were landmarks in microbial engineering. Improvements in the ability to reprogram plants will impact a wide range of industries including textiles, fuels, sugars, fine chemicals, drugs and food. The publication of a common genetic syntax that enables the exchange of standard DNA parts for plants and other eukaryotes was a major outcome of the first year of OpenPlant (Patron *et a*l., 2015). The standard has been ratified by an international consortium of scientists, and subsequently we have seen it accepted as the new Phytobrick standard for the sharing of plant DNA parts via the Registry of Biological Parts and the establishment of an inaugural Plant Award at in the 2016 iGEM competition. In the

third year of OpenPlant, we saw the development of the Loop assembly protocol for fast and efficient construction of PhytoBrick compatible Level zero DNA parts into large scale genetic assemblies. We now see the standard being applied to the production of parts for genome editing, the engineering of novel traits and to enable coordinated development of supporting hardware and software for bioengineering. This year has seen a major step forward with the publication of the OpenMTA in Nature Biotechnology, it's official worldwide release, and imminent adoption as a standard option at Addgene.

The social acceptance of genetic modification in field-grown crop plants remains a significant barrier to the adoption of plant synthetic biology in the UK. OpenPlant research, public engagement and outreach efforts promote (i) models for decentralised ownership and control of key technologies, (ii) use of *cis*-genics and precision gene editing technologies, (iii) development of new crop traits with improved properties, sustainable production, resource management and environmental impact, and (iv) aid international development and technical exchange for agriculture and sustainable land use.

FUTURE PLANS

- Consolidate chromosome scale map of the Marchantia genome
- Integrate single-cell transcriptomic data with cell-type data
- Characterise transcription factor promoter-fusions as cellular markers for Marchantia development
- Test promoter dynamics in surgically excised gemma tissues
- Adopt machine-learning approaches to build models for genetic-cellular control of growth
- Apply gene editing tools for reprogramming growth
- Further develop DNA tools for high-level expression in chloroplasts
- Continued development of trait-based technologies
- International implementation of the OpenMTA agreement
- Build a network of UK-African scientists for Synthetic Biology
- Look for opportunities to promote open technologies by technical exchange and resource sharing in Latin America and Africa
- Develop cell-free systems for implementation in open curriculum development