Post-doctoral Research Fellowship

School of Biological Sciences

The University of Edinburgh

Hetero-trans-β-glucanase (HTG), a unique cell-wall remodelling enzyme from *Equisetum*: action and potential to enhance mechanical properties of cereals

The Edinburgh Cell Wall Group, Institute of Molecular Plant Sciences, The University of Edinburgh, Daniel Rutherford Building, The King's Buildings, Max Born Crescent, Edinburgh EH9 3BF, UK

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Co-PI: Prof. Andrew Hudson (IMPS)

Industrial collaborator: Dr Frank Meulewaeter, Bayer CropScience, Gent, Belgium

Start date: as soon as possible.

End date: 31 December 2018.

Non-technical summary

BACKGROUND AND PURPOSE

The School of Biological Sciences has recently been very successful with applications for BBSRC funded Industrial Partnership Awards (IPAs), which are a useful mechanism to consolidate collaborative relationships with industry. The company — Bayer CropScience in our case — commits at least 10% of the total grant applied for in cash. BBSRC IPA Awards help to develop research projects with leading multinationals.

The Edinburgh Cell Wall Group (ECWG), led by Prof. Stephen Fry, recently discovered a unique enzyme (HTG or hetero-trans-β-glucanase), found only in an isolated group of non-flowering plants, the horsetails (*Equisetum* spp.). Flowering plants lack HTG even though their cell walls contain the hemicellulose (polysaccharide) molecules which, at least in the test-tube, HTG is capable of cutting and re-joining. Indeed horsetail HTG is

the only known enzyme from any living thing that can 'cut and re-join' molecules of cellulose, the major constituent of plant cell walls.

The ECWG will use the IPA to aim to discover what the benefit of HTG is for horsetail plants, the full range of 'cutting and re-joining' reactions that HTG can achieve, and what might happen if HTG is artificially transferred from *Equisetum* to crop plants. In theory at least, HTG might endow flowering-plant crops (e.g. wheat) with the ability to strengthen their stems and increase their resistance to storm damage (called lodging), which can cause serious loss of crop yield. Normally, cereal varieties with stronger stems suffer less lodging but this is often as the expense of lower grain yield. The question is whether artificially giving cereals HTG may confer similar strengthening without compromising the harvest. Our industrial collaborators (a major multinational crop science company) are interested in trying this out ‘in the field.’ Modifying cereals in this way would benefit plant breeders and farmers, as well as the general public, by improving the reliability of grain production in a changing climate as storms and heavy rains become more frequent.

OBJECTIVES AND EXPECTED OUTCOMES

Remarkably, horsetail HTG is the only known enzyme from any living thing that can 'cut and re-join' molecules of cellulose, the major constituent of plant cell walls. It can graft a cellulose chain onto a chain of a different cell-wall building material called xyloglucan. HTG can also graft chains of a third such polysaccharide (MLG or mixed-linkage glucan) onto xyloglucan. HTG can thus create cellulose-to-xyloglucan and MLG-to-xyloglucan linkages. The resulting 'hybrid' polymers are thought to strengthen horsetails. We will discover exactly when and where HTG is produced, and such linkages are formed, in horsetails. This will potentially give clues about HTG's natural roles. We will also discover what new reactions HTG can catalyse when mixed in the test-tube with diverse plant cell-wall polysaccharides. This may afford new 'hybrid' polymers, which when scaled up may be commercially valuable new materials. To further our fundamental knowledge of HTG, we will also use site-directed mutagenesis to investigate which of the enzyme's amino acids are important for its ability (in the test-tube) to re-configure cellulose and MLG.

A major part of this project involves artificially introducing the horsetail's HTG activities into flowering plants, including both dicotyledons and cereals, and measuring the consequences. Our industrial collaborators (Bayer CropScience) will do this work in the case of wheat. We predict that any crop plants genetically transformed in this way will be able to create cellulose-to-xyloglucan linkages in their cell walls, and that cereals (which, unlike dicots, possess MLG as well as cellulose and xyloglucan) will in addition be able to make MLG-to-xyloglucan linkages. We will test these predictions experimentally. We will also test whether the HTG-endowed flowering plants are stronger, and whether they have an altered shape or size. We will quantify the plants' mechanical strength by measuring the force required to bend or break their stems. Any

changes to the molecular architecture a plant's cell walls are likely to affect its growth and strength because of the pivotal roles that cell walls play in dictating these features.

BENEFICIARIES OF THE PROJECT

Cereal varieties with stronger stems often suffer less lodging, but such strengthening is usually achieved by the plant growing thicker stems at the expense of lower grain yield. Artificially giving cereals HTG may form novel inter-polymer linkages in the cell wall and confer similar strengthening without significant increases in stem biomass and thus without compromising the harvest. Modifying cereals in this way would benefit plant breeders and farmers, as well as the general public, by improving the reliability of grain production in a changing climate as storms and heavy rains become more frequent. In addition, increasing knowledge of HTG's ability to reconfigure biomass materials, especially cellulose (the world's most abundant organic substance), offers biotechnologists novel opportunities to create new materials (e.g. for specialist papers and medical applications) via non-polluting 'green' processes.

Technical summary

Plant cell walls contain transglycanase activities that 'cut and paste' hemicelluloses — chains of xyloglucan, xylan, mannan and mixed-linkage β-glucan (MLG). Classic xyloglucan-acting transglycanases (XTHs, exhibiting XET activity) potentially contribute to wall architecture. Until recently, no cellulose-acting transglycanase was known. However, we recently discovered and sequenced HTG, an *Equisetum* enzyme that preferentially catalyses hetero-transglycosylation with cellulose or MLG as donor substrate and xyloglucan as acceptor (Simmons *et al.*, 2015). HTG has three enzymic activities known to date:

- CXE (cellulose : xyloglucan endotransglucosylase)
- MXE (MLG : xyloglucan endotransglucosylase)
- XET (xyloglucan : xyloglucan endotransglucosylase)

HTG's ability to make cellulose–xyloglucan and MLG–xyloglucan bonds may be valuable, both for functionalising biomass post harvest and for strengthening living crops. We will explore HTG's natural role in *Equisetum*, further define its *in-vitro* and *in-vivo* catalytic repertoire, and test the effects of an *HTG* transgene on the growth, morphogenesis and mechanical properties of *Arabidopsis*, wheat and maize. Wild-type angiosperms lack HTG but possess its substrates (cellulose and xyloglucan in dicots; cellulose, xyloglucan and MLG in cereals), so we predict that transgenic HTG will act in crops. We will test this prediction. Effects of heterologous HTG may also add to our fundamental understanding of wall architecture. By site-directed mutagenesis, we will test the contribution of what are predicted (by 3D modelling) to be the 3 key amino-acid substitutions that presumably 'converted' an *Equisetum* XTH into HTG. The results may suggest whether it is feasible to confer 'HTG' on crop plants through directed mutation of endogenous XTHs. In collaboration with Bayer CropScience we will test whether HTG expression in crops minimises lodging by strengthening cell walls via

cellulose–xyloglucan or MLG–xyloglucan linkages that have until now been confined to *Equisetum*. The project will exploit our unique expertise and recent discovery of HTG to perform proof-of-concept studies into crop improvement and to explore HTG's potential for post-harvest synthesis of novel bio-materials (e.g. speciality papers) via environmentally friendly biotechnological (synthetic biology) approaches.

The Hudson and Meulewaeter labs are generating transgenic angiosperms and *E. coli* expressing the *HTG* gene from *Equisetum fluviatile*, and these as well as the HTG enzyme itself are being characterised in the ECWG (Fry lab). Methodology employed by the new postdoc in the ECWG will include:

- *in-situ* detection of CXE, MXE and XET enzyme action by *in-vivo* radiolabelling and fluorescence microscopy;
- enzymologically characterising HTG by novel enzyme assays, mainly involving *in-vitro* radiolabelling;
- localising HTG protein in *Equisetum* and *HTG*-transformed angiosperms, by immunocytochemistry;
- characterising the growth rate, morphology and histology of *HTG*-transformed angiosperms;
- producing HTG protein samples with different degrees of *N*-glycosylation, and characterising of the effect of glycosylation state of HTG on its enzymic activities.

The job does not involve conducting work on DNA or RNA; these aspects are covered by the Hudson and Meulewaeter labs.

THE PROJECT IS BASED ON THE FOLLOWING MAIN PUBLICATIONS

T.J. Simmons,* K.E. Mohler,* C. Holland,* F. Goubet, L. Franková,† D.R. Houston, A.D. Hudson, F. Meulewaeter, S.C. Fry (2015) Hetero-trans-β-glucanase, an enzyme unique to *Equisetum* plants, functionalises cellulose. *Plant Journal*, **83**, 753–769.

L. Franková,† S.C. Fry (2013) Darwin Review: Biochemistry and physiological roles of enzymes that 'cut and paste' plant cell-wall polysaccharides. *Journal of Experimental Botany*, **64**, 3519–3550.

*Former PhD students in Fry lab.

†Post-doc in Fry lab.

Organisational background

The project began on 1 January 2016, and is scheduled to run until 31 December 2018. The Edinburgh team currently comprises:

- Prof. Stephen C. Fry (PI)
- Prof Andrew Hudson (co-PI)
- Dr Lenka Franková (postdoc)
- Ms Amy Wallace (technician in Fry lab)
- Ms Erica de Leau (technician in Hudson lab)

With effect from 1 January 2017, Dr Franková has been appointed to a Lectureship at Newcastle University, and the postdoc position will be vacant. However, funding permits the new postdoc to be appointed as soon as possible; it is hoped that his/her appointment will overlap with Dr Franková's and run until 31 December 2018.

HOW TO APPLY

Please visit the University of Edinburgh jobs website (https://www.vacancies.ed.ac.uk/) search for vacancy reference 037457, and apply online through that website.

Informal enquiries can be made to Professor Stephen C. Fry F.R.S.E., The Edinburgh Cell Wall Group, Institute of Molecular Plant Sciences, The University of Edinburgh, Daniel Rutherford Building, The King's Buildings, Max Born Crescent, Edinburgh EH9 3BF, UK.

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