# Plant Cell Walls

*ARC Centre of Excellence*

THE UNIVERSITY *of* ADELAIDE

THE UNIVERSITY OF QUEENSLAND AUSTRALIA

THE UNIVERSITY OF MELBOURNE

# The Scientific Question

How do plants regulate the synthesis, assembly and re-modelling of their cell walls during normal development, and in response to biotic stresses?

## The Vision

The Centre will build on existing national strengths in the field of fundamental cell wall biology to:

- generate scale and focus at the scientific, technical and training levels

- enhance plant biotechnologies to underpin Australian cereal industries valued at over $8 billion p.a., associated food industries valued at about $40 billion p.a., and massive emerging industries related to the production of renewable transport fuels, biomedicines and biocomposites

### Why grasses?

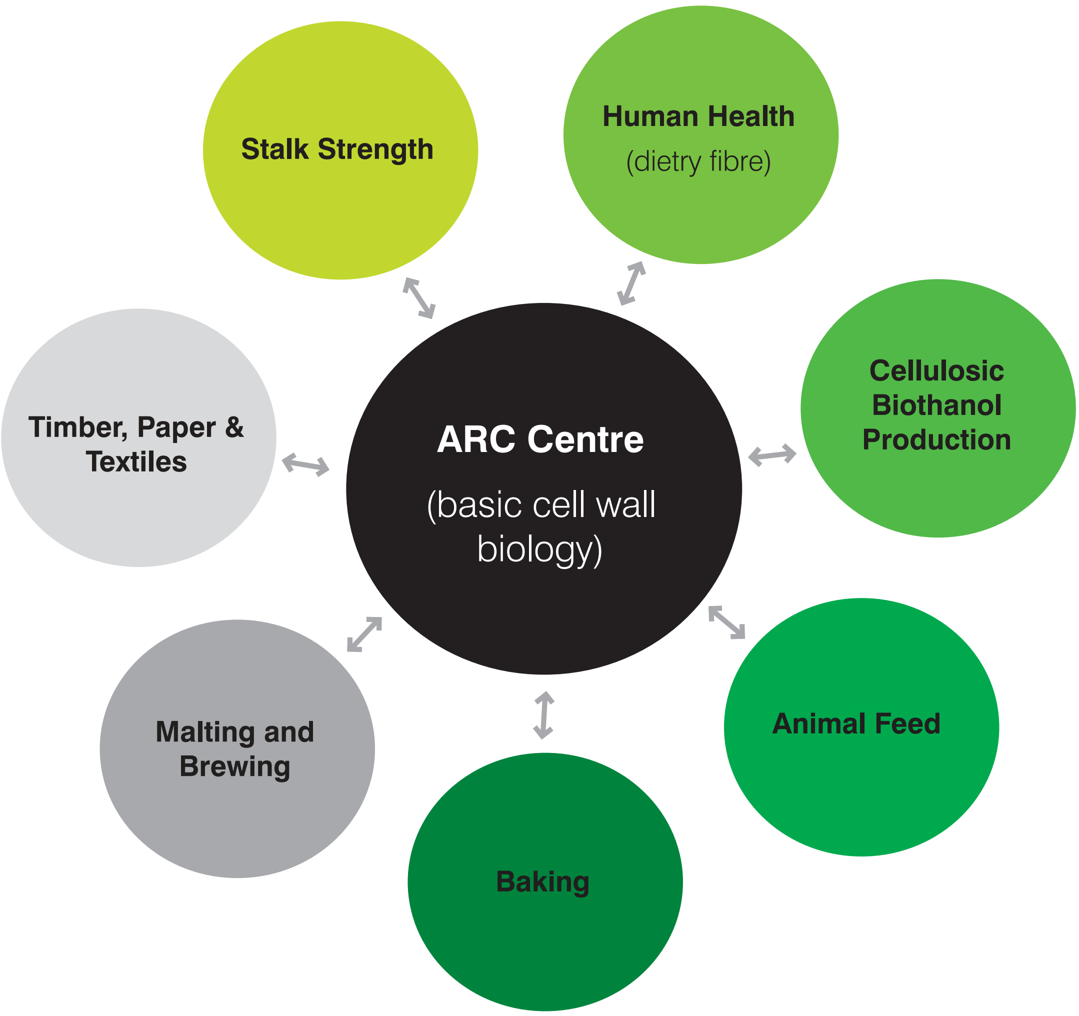
Grasses represent the most important family of plants for humans. Foods made from rice, wheat, maize, sorghum, barley, millets and sugar cane account for a high proportion of global caloric intake. Forage and fodder grasses support the production of domesticated livestock, while other perennial grasses show great promise as biomass energy crops.

### Why cell walls?

Cell walls determine the quality of most plant-based products used in modern human societies. Textural, nutritional and processing properties of plant-based foods are heavily influenced by wall properties. As the largest source of renewable carbon, plant cell walls have a critical future role in transport fuels, food security, functional foods to improve human health, and as raw materials for industrial processes

## Our Mission

To advance fundamental scientific understanding of plant cell wall biology to enable sustainable biomass production for food security, human health and energy biomass conversion.



**Media Release**

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ARC Centre of Excellence

# $31 million biotech centre to benefit crops, food, energy

**Friday, 16 July 2010**

Australia's crop and food industries will benefit from a new $31 million biotechnology Centre of Excellence to be headquartered at the University of Adelaide's Waite Campus.

The University has today been awarded $19.25 million in federal funding from the Australian Research Council (ARC), with an additional $12 million of support from partner institutions.

The University's new ARC Centre of Excellence in Plant Cell Wall Biology will build an international team of researchers with strong industry links.

Their research will significantly enhance biotechnologies that underpin Australian crop industries valued at more than $8 billion per annum, associated food industries valued at $40 billion per annum, and emerging industries related to renewable transport fuels and biomaterials.

The Centre of Excellence will be led by the University of Adelaide and involves collaboration with the universities of Melbourne and Queensland, and with major research institutions and industry partners in Australia, Scotland, Sweden, Germany and the United States.

The Director of the new Centre of Excellence is Professor Geoff Fincher, who has many years of experience in agricultural and biotechnology research. He will work with senior colleagues Dr Rachel Burton (University of Adelaide), Professor Tony Bacic (University of Melbourne) and Professor Mike Gidley (University of Queensland).

"Plant cell walls contain components that are of major interest for renewable energy production, for the food industry, and for the pulp and paper industries," Professor Fincher says.

"Once the plant has died, most of what is left is material from the cell walls. So there are opportunities to utilise this material in new ways and to add further value to Australia's crop industries.

"The central role of plant cell walls as renewable sources of transport fuels, as new food products, and as a source of raw materials for industrial processes is a huge driver for international research.

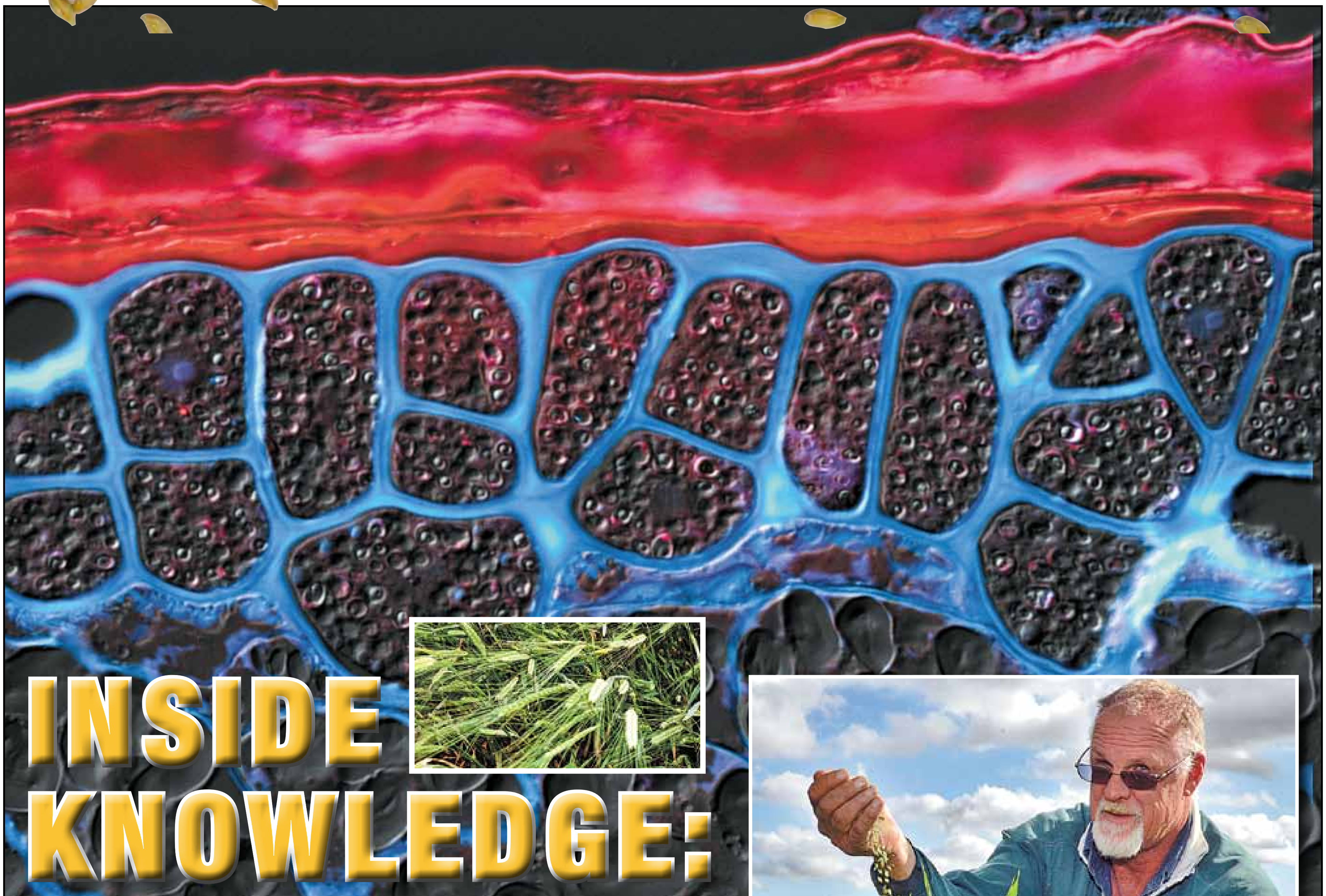
"When one considers that about 180 billion tonnes of the cell wall component, cellulose, is renewed on earth every year, it is not surprising that this part of the plant is attracting attention for renewable fuel production. Because humans cannot digest cellulose, the production of cellulosic residues for biofuels does not compete for human food production."

University of Adelaide Vice-Chancellor and President Professor James McWha says the new ARC Centre of Excellence builds on the unique, collaborative research strengths in agriculture and biotechnology at the Waite Campus.

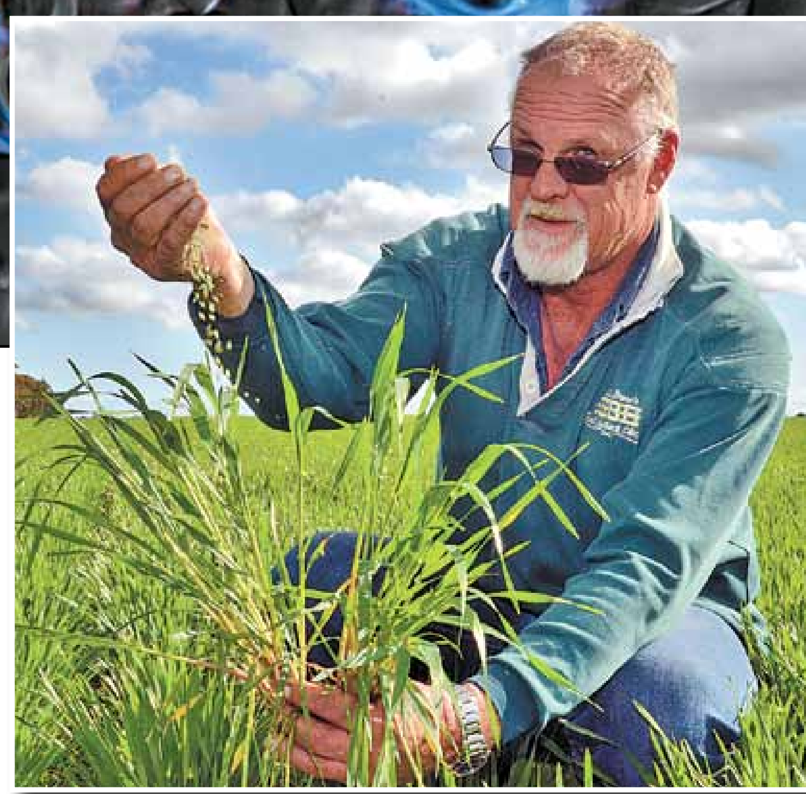
"This world-class centre will be the focal point for the development of new technologies and processes. It will play a key role in enhancing the University's reputation for agricultural research and training, and it will boost our already significant contribution to industry and the community," Professor McWha says.

The University of Adelaide is a key partner in the ARC Centre of Excellence for Particle Physics at the Tera-Scale and the ARC Centre of Excellence for the History of Emotions, which were also announced today.

# THIS IS A GRAIN OF BARLEY:



# INSIDE KNOWLEDGE:



LONG Plains farmer Cliff Fabry with growing barley – and the result.

LEFT: cellulose in the cell wall of vascular tissues found in barley stem sections. Images supplied by Ashley Tan and (above) Dr Matthew Tucker.

## Researchers are hoping it will change the world

THIS is what the inside of a grain of barley looks like. It's a section of a mature barley grain stained with calcofluor (fluorescent dye) to highlight the endosperm cell walls (blue) and seed coat (red). Most would recognise the green stuff, inset.

The barley research – and much more – is taking place at a major new research centre launched this month at the University of Adelaide's Waite Campus.

The ARC Centre of Excellence in Plant Cell Walls is a $32 million biotechnology centre that looks at the fundamental role of cell walls (biomass) in plants – in particular grasses and cereal crops – and how they can be better utilised for the benefit of human societies to bring improved nutrition for billions of people around the world and the development of clean, green biofuels.

Barley, wheat, rice and model grasses will be the focal points of this research.

The centre is hoping to discover ways of altering the macromolecules inside the cell walls to maximise their potential for reducing the risk of serious human diseases (through altering the soluble fibre levels in grain) and to benefit industry in diverse areas such as bioethanol production, malting processes for beer production, and pulp and paper manufacture.

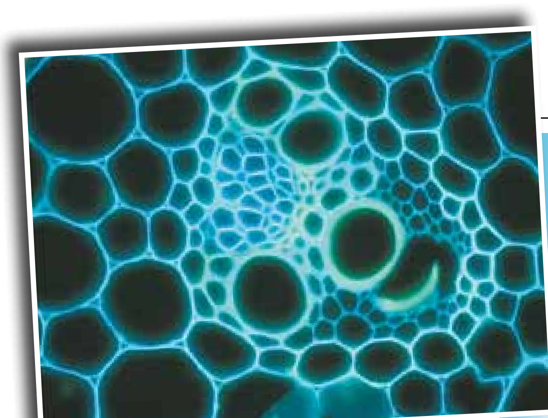
Headquartered at the University of Adelaide, the centre involves collaboration with the universities of Melbourne and Queensland and has other major research and industry partners in Scotland, Sweden, Germany and the United States.

The centre has been established with $20.5 million in Federal cash funding from the Australian Research Council (ARC) and an additional $12 million of support from partner institutions, and the State government.

Director of the new centre is Professor Geoff Fincher, who has more than 37 years experience in plant and agricultural science.

ARC chief executive officer, professor Margaret Sheil said the centre would play a critical role in defining the fundamental science that controls cell wall biology in plants, including important crop species.

"Fundamental scientific discoveries will



inevitably point the way towards new technologies that will underpin Australian crop industries, which are valued at more than $8 billion per year, associated food industries valued at $40 billion per year, and the rapidly growing biofuels and biomaterials industries," Professor Sheil said.

"The centre brings together a team of internationally regarded scientists, with research and industry partners from around the globe.

These include: Scotland: University of Dundee and the James Hutton Institute; Sweden: Biomime and KTH (Royal Institute of Technology of Sweden); Germany: Leibniz-Institute of Plant Genetics and Crop Plant Research) and the United States: Energy Biosciences Institute), Arcadia Biosciences Inc and DuPont-Pioneer.

## WHAT SCIENCE IS SEEKING:

■ CELL walls play a number of key roles in plants, such as providing strength and flexibility for the plant; allowing water into the cell and being waterproof when needed; providing a physical barrier to invasion by pests; allowing changes during the growth of the plant; and responding to stresses on the plant.

A key component is cellulose, one of the most abundant molecules on the planet – an estimated 180 billion tonnes of cellulose is produced by plants each year. Cellulose is made up of glucose (sugar) molecules, which link up to form polysaccharides.

The centre's researchers will work to better understand the polysaccharides in plant cell walls, aiming to alter the polysaccharides in grasses and cereals to produce a range of benefits for human health and industry.

These include:

■ **Improved fibre in grains:**
Polysaccharides, known as beta-glucans, are important to dietary fibre because they are not digested in the small intestine. Improving fibre levels in grains such as wheat and rice could reduce the risk of colorectal cancer, cardiovascular disease, type 2 diabetes and a number of other serious diseases for billions of people.

■ **Improved animal nutrition:**
While beta-glucans might be beneficial in dietary fibre in humans, for production animals such as chickens and pigs, it's desirable to have lower beta-glucan levels in feed.

■ **Malting and brewing:**
Glucans in grain are also undesirable for maltsters and brewers, because beta-glucans contribute to beer "haze". The cell wall group in Adelaide has a collaborative project with Viterra in this area.

■ **Bioethanol production:**
Bioethanol is a renewable fuel that is produced by the fermentation of sugars. Grasses are the major source of biomass for bioethanol production.

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# Professor Geoff Fincher – Centre Director

Key requirements for the role of Centre Director in the ARC Centre of Excellence for Plant Cell Wall and for other scientific leadership roles include an ability to provide ongoing intellectual input during the development, implementation and revision of research strategies, to put in place robust governance structures, to focus research activities on the goals of the Centre while maintaining sufficient flexibility to pursue promising but unexpected new directions, to manage scientists, students and general staff in an equitable and encouraging way, to provide a highly stimulating and positive research environment, to ensure that both intellectual and research infrastructure are developed in line with emerging technologies, and to forge national and international collaborative networks that will bring enhanced multidisciplinary capabilities to the Centre.

In terms of building and leading large research teams, Professor Fincher was the Head of the Department of Plant Science during the period 1993-2002. By 2002, the Department of Plant Science had an annual DETYA core budget of more than $2 million, 15 lecturing staff, more than 150 staff and postgraduate members overall, and a research income of more than $12 million. Subsequently he was the Director of the GRDC's Cereal Functional Genomics Centre and he was a Chief Investigator (CI) on the original application for the ACPFG, where he remained the Deputy CEO and chaired the Executive Management Group until the end of 2010. Professor Fincher is also a CI on the $52 million Australian Plant Phenomics Facility, where he serves on the Executive Management Committee. He is also the Director of the new $3.55 million CSIRO Food Futures Cluster on Healthy Cereal Complex Carbohydrates. For the last seven years Professor Fincher has been Director of the Waite Campus of the University of Adelaide, although this appointment is now completed.

In addition to meeting these leadership requirements, Professor Fincher continues to contribute strong scientific input to the research activities of the Centre and puts in place mechanisms to ensure that this occurs. CIs Bacic, Burton and Gidley are formally responsible for the three research programs, but he participates in workshops and teleconferences involving the national and international partners, and in the formal scientific meetings that convene three times a year.

Professor Fincher sees the efficient management of our international partners as a major responsibility of the Centre Director. In particular, he assists the international PIs with applications for expanded funding and establishing a series of face-to-face workshops, together with regular teleconferences, to ensure that the partner organizations are and remain fully integrated with the ARC Centre.

All involved in the Centre have contributed to a range of areas critical to Australia's scientific performance in the international arena. These areas include the establishment and management of large scale, nationally collaborative research groups, the training of scientists to the highest international standards, the application of emerging technologies for the innovative solution of problems facing rural communities and industries, the identification and acquisition of large scale infrastructure essential for advancing Australia's ability to compete in international marketplaces, and the identification of solutions to future challenges related to water use efficiency and climate change.

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# Program 1 – Molecular Genetics Of Wall Synthesis
# Dr Rachel Burton, University Of Adelaide

Objective:

Define regulatory processes that control cell wall polysaccharide biosynthesis in model grasses; commercially applicable to important cereals and grasses

Specific Aims:

Build on previous projects to:

- dentify gene families and enzymes involved in wall poly-saccharide synthesis, nremodeling and degradation in grasses
- analyse gene and protein networks implicated in wall poly-saccharide metabolism in cereals and grasses more generally.

**Experimental Approaches**

- apply forward genetics technologies to identify and characterize genes and their products involved in the regulation of wall polysaccharide and glycoprotein synthesis in grasses, using:
- barley diversity collections and association (linkage disequilibrium) mapping methods (with Al Mather)
- high throughput phenotyping assays for cellulose, lignin and other wall constituents
- comparative genomics and positional cloning to identify regulatory genes
- proof-of-function techniques for candidate genes identified through this approach.
- use reverse genetics approaches to investigate the roles of specific genes and transcription factors that we have previously implicated in wall polysaccharide synthesis, and ChIP-sequencing to analyze protein interactions with DNA
- characterize candidate genes for the synthesis of (glucurono)arabinoxylans in the grasses
- screen barley and maize microRNA (miRNA) gene libraries for miRNAs that might target genes that have been implicated in cell wall metabolism in the grasses.
- express selected genes in heterologous systems to test the biological relevance of protein and enzyme interactions implied by gene networks and transcriptional co-regulation
- examine evolution and function of the conserved cellulose synthase-like (Csl) gene clusters by:
- comparing conserved sequences, marker motifs and exon-intron arrangements to identify potential recombination, deletion or insertion sites that led to the evolution and functional diversification of Csl genes
- testing hybrid gene constructs, based on these results, in heterologous expression systems
- defining Csl copy number variation in grasses and relating any variation to differences in wall polysaccharide structure or content in grain or vegetative tissues.

**Potentially Transformational Areas of Research**

- defining the genes and enzymes involved in (glucurono)arabinoxylan biosynthesis
- defining the genetic recombinations and other events leading to the molecular evolution of (1,3;1,4)-ß-glucan synthase genes in the grasses
- defining the genetic and biochemical control of wall polysaccharide fine structure.

# Program 2 - Cell Wall Organization And Molecular Architecture
# Professor Mike Gidley, University Of Queensland

Objectives:

- Define relationships between the chemical fine structure of wall polysaccharides and their physicochemical properties
- Define the nature of covalent and non-covalent linkages between key wall constituents
- Relate molecular architecture to macroscopic properties.

Specific Aims:

- define fine structures & physicochemical properties of major wall polysaccharides and link with biosynthetic mechanisms
- define enzymology and genetics of the formation of covalent lignin-carbohydrate cross-links
- characterize putative linkages between cellulose and other polysaccharides in walls from grasses
- define molecular size distribution and solution properties
- construct and characterize defined, cellulose-based composites with other wall polysaccharides and lignin
- define compositional/property gradients across single walls
- identify motional rigidity of polymer segments within hydrated cell walls, and relate to mechanical properties
- define material properties of walls from nano- to macro-scale

**Experimental Approaches**

- **Polymers and Composites:** Construct cellulose-based composites with polysaccharides and/ or lignin by fermentation of Gluconacetobacter xylinus, as assembly, mechanical and re-modeling mimics for grass cell walls, as pioneered by CI Gidley for non-grass walls. We will:
  - Define molecular interactions between polysaccharides/lignin and cellulose, architectural principles of model wall construction, and relate to materials properties (with AI Stokes)
  - dentify structural/microscopic/spectroscopic and/or mechanical signatures; use as probes to diagnose wall structure/properties in microscopically-defined locations
  - Define effects of molecular size and fine structure of wall polysaccharides on properties in solution (with AI Gilbert) and in cellulose composites.
- **Cell Walls in situ:** Using plant genotypes with quantified wall composition variations (selected from those available through the Centre), relationships between molecular structure, localized wall architecture / interactions, and materials properties will be defined. We will:
  - Characterize material properties at cell wall, isolated cell, and separated tissue levels using nano- and micro-rheology and tribology techniques (with AI Stokes – see section G)
  - Identify structural/microscopic/spectroscopic signatures within walls, cells, and tissues using FTIR microspecroscopy (Australian synchrotron), Raman nanospectroscopy (with PI Somerville), and immunohistochemistry, and relate to materials properties
  - Define effects of molecular structure and wall architecture on re-modeling and degradation

**Potentially Transformational Areas of Research**

- defining how the major components are organised in the wall, at a precise molecular level
- defining how changes in fine structure of wall constituents obtained from different genotypes affect physicochemical properties and interactions with other wall components
- producing first 'molecules to materials' model for cell walls, validated at nm-cm length scales
- manipulating enzymes involved in covalent cross-linking wall polysaccharides and in polysaccharide-lignin linkages, to enhance conversion processes during bioethanol production

# Program 3 – Cell Biology And Biochemistry Of Walls
# Professor Tony Bacic, University Of Melbourne

Objectives:

- sub-cellular sites of wall polymer synthesis
- mechanisms of assembly
- function of membrane rafts
- 3D structures of synthase complexes in the membrane the nature of the interaction between the wall-plasma membrane-cytoskeleton continuum and re-modeling of wall polysaccharides during growth and biotic stress.

Specific Aims:

- define the intracellular pathway of assembly of key wall polymers with a view to identifying enzymes, cofactors and accessory proteins, together with post-translational modifications, that are involved in their assembly and vesicular transport to the cell surface
- isolate membrane rafts and analyse their protein and lipid complements, with a view to identifying important glycosyltransferases, together with other proteins involved in wall synthesis
- define roles of lipid raft-like structures in wall assembly
- characterize nascent wall polysaccharides and/or oligosaccharides associated with membrane rafts
- nvestigate the role of the wall as a short term energy store through integrated transcriptomics/metabolomics
- define interactions occurring at the wall-plasma membrane-cytoskeleton continuum during growth and pathogen infection to understand how plants sense their environment and initiate signal transduction cascades.

**Experimental Approaches**

- CI Bacic and other Centre members have extensive expertise in:
  - the biochemistry and cell biology of polysaccharide and proteoglycan biosynthesis
  - structure determination in plant systems, including wall and organelle isolation
  - immunocytochemistry, fluorescence microscopy, EM tomography
  - metabolomics, proteomics, and the assay and purification of enzymes
- through our PIs we have access to experimental expertise in:
  - isolation of polysaccharide synthase complexes and membrane rafts (PI Bulone)
  - mutant identification and analysis of cell surface sensing molecules (PI Somerville)
  - high-throughput screening of disease resistance plant pathology, and plant-pathogen interaction microarrays (PIs Schweizer and Waugh)
  - lignin biology (PIs Halpin and Waugh).
- this expertise is supported by state-of-the-art infrastructure available in our own laboratories, or accessible through collaborating organizations and/or major national infrastructure facilities.

**Potentially Transformational Areas of Research**

- integrating high-throughput 'omics' technologies with cell biology and biochemistry
- defining the subcellular pathway of assembly and transport of secreted macromolecules
- characterization of membrane complexes in non-cellulosic polysaccharide biosynthesis
- defining the nature of the wall-plasma membrane-cytoskeleton continuum in plants and its role as a signal transducer in normal growth and during biotic stress
- integrating metabolism with sugar sensing and carbon allocation by defining a possible role for wall polysaccharides in the short term storage of metabolizable sugars in the grasses
- dentifying novel cell wall-related genes that contribute to non-host pathogen resistance.

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# International Partners

**Professor Vincent Bulone**
Royal Institue of Technology (KTH), Sweden & Biomime

- is Director of the $12m Center for Biomimetic Fiber Engineering; mimicking evolutionary biological systems for new surface and fiber chemistries
- is an expert in the isolation of membrane rafts (Program 3)
- is one of few to express cDNAs for polysaccharide synthase enzymes and shown polysaccharide biosynthesis in vitro; invaluable expertise for Program 1
- will submit joint applications with the ARC Centre to the Swedish Foundation for Strategic Research, the Swedish Research Council and other funders

KTH VETENSKAP OCH KONST

PDC Center for High Performance Computing

BIOMIME

**Professor Claire Halpin**
University of Dundee, UK

- is the Director of the Cell Wall Lignin program within the £26 million BBSRC Sustainable Bioenergy Centre at the University of Dundee (UK)
- is expert in the biochemistry and cell and molecular biology of lignin synthesis
- will collaborate on lignin metabolism in Programs 1 and 2
- will apply for additional funding from U.K. and European agencies for expanded joint projects with the Centre on the lignin-polysaccharide interface

University of Dundee

**Dr Vic Knauf**
Arcadia Biosciences Inc., USA

- is Chief Scientific Officer with Arcadia Biosciences
- has extensive experience in developing and managing multi-million dollar commercial biotechnology projects
- brings specialized expertise in TILLING in wheat, and sorghum transformation
- has committed $360,000 cash to the proposed ARC Centre

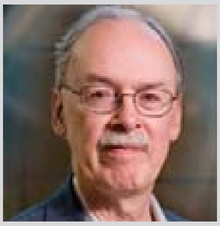
Arcadia BIOSCIENCES

**Dr Patrick Schweizer**
Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Germany

- is leader of the Plant Genome Resources Center at the $54M p.a. Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) in Germany
- will collaborate with Program 3 to advance knowledge in plant-microbe interactions, and hence in crop productivity and food security
- will apply for new joint grants through the German GABI program and the EU

IPK GATERSLEBEN

# International Partners



**Professor Christopher Somerville**
Energy Biosciences Institute, USA

- is Director of the Energy Biosciences Institute based at UC Berkeley; the largest biofuels research centre in the world, focusing on the generation of bioethanol from lignocellulose derived from cell walls of perennial grasses
- is supported in part by a USD$350 million grant from BP
- sees the ARC Centre as a potential Australian partner for EBI's global focus on alternative fuels from perennial grasses, consistent with our focus on grasses
- will allocate $1.75 million to joint projects in the Centre, over 7 years

Energy Biosciences Institute

**Dr Scott Tingey**
DuPont Agricultural Biotechnology, USA

- is Director of Genetics at DuPont-Pioneer, which recently announced a $40m investment in advanced plant genetics to boost agricultural productivity
- has a long term research relationship with CIs Bacic and Fincher, through ACPFG funded projects and ARC LP grants
- has made available maize genome facilities, microarray analyses and NGS
- will seek internal funding, $160k p.a., from DuPont-Pioneer for 2012-2014

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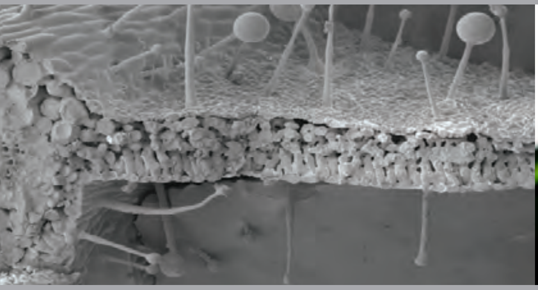
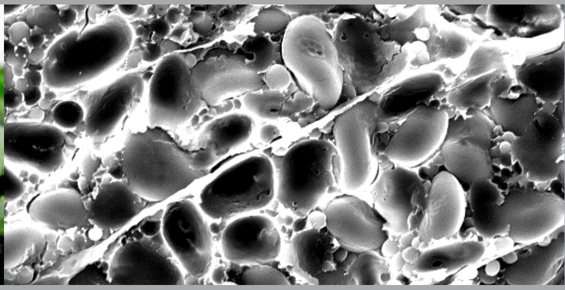
**Professor Robbie Waugh**
The James Hutton Institute, UK

- eads a department of 90 scientists at the Scottish Crop Research Institute, with an annual budget of approx. £2 million for barley alone
- will provide the most comprehensive barley genetic resources in the world
- will seek funding from UK and European sources for expanded projects on the genetics and evolution of polysaccharide synthases in the grasses, and on changes in cell walls during plant-pathogen interactions.

The James Hutton Institute

Leading the way in the international research of **Plant Cell Walls**





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Australian Government
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