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Studies of Barley Cell Wall Synthesis in Coleoptiles and Protoplasts using a Proteomics Approach.

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Abstract

Cell walls are central to the growth and development of cereals and are important contributors to many quality characteristics of cereal grains. Subtle differences in the fine structures of major wall polysaccharides can have dramatic effects on the functional/rheological properties of cereal-based products.

Cell wall synthesis is being studied by isolating the sub-cellular compartments involved in the synthesis and secretory pathway (endoplasmic reticulum, Golgi apparatus, plasma membrane and cell wall) in coleoptiles and protoplasts. Proteins involved in cell wall synthesis are identified by studying the differential expression of proteins at various time points and analyzing the products using 2D SDS-PAGE electrophoresis and mass spectrometry.

Introduction

Proteomics is the systematic analysis of the protein population in a tissue, cell or sub-cellular compartment. It involves protein separation, mass spectrometry (MS) and bioinformatics.

This can be compared to genomics, which aims to understand the function of genes using bioinformatics to study DNA sequences stored in sequence databases and gene expression profiles, and mutants.

Proteomics is still in its infancy but is likely to become an active field with a large impact on plant biology (van Wijk, 2001).

Between 1,000 and 2,000 proteins can be routinely separated on 2D gels. There are a number of approaches but in summary, individual proteins are identified based upon a

combination of amino acid composition, peptide mass spectrometry fingerprinting, N-terminal sequence, Mr by MS and pI data, which are then analysed through appropriate protein databases. These methods are effective for identifying many proteins, although large molecular weight and insoluble proteins, such as integral membrane proteins, are harder to analyze.

Proteins involved in wall synthesis are synthesized and transported via the protein secretory pathway. The Golgi apparatus is a central organelle in this pathway. Proteins assembled in the lumen of endoplasmic reticulum (ER) are passed through the Golgi and transported to the vacuole, plasma membrane, or cell wall (Wee *et al*. 1998). The enzymes that synthesize a wide range of carbohydrate structures are located in membranes of the Golgi apparatus and plasma membrane. For example, pectins and non cellulose polysaccharides, which are cell wall matrix polysaccharides, are manufactured by transfer of sugars from sugar nucleotide precursors by glycosyltransferases present in the ER (Gibeaut and Carpita, 1994).

The aim of this study is to identify proteins and pathways involved in cell wall synthesis in coleoptiles and protoplasts using reproducible 2D SDS-PAGE, and sensitive mass spectrometry protein analysis techniques.

Materials and Methods

Plant Material

Barley (*Hordeum vugare* cv. Schooner) cell suspension cultures were grown on shakers at 25_C. Protoplasts were obtained 4 days after sub-culturing using the methods described by Shea *et al*. (1989) and Singh *et al* (1997). Barley (*Hordeum vugare* cv. Sloop) certified and treated with fungicide *Zea mays* (DeKalb) was grown in saturated vermiculite to produce rapidly growing coleoptiles.

Isolation of Subcellular Compartments

Protoplasts and coleoptiles were ruptured by grinding in liquid nitrogen for five minutes. Endoplasmic reticulum, Golgi apparatus and plasma membrane were isolated from the homogenized protoplasts and coleoptiles by centrifuging membranes onto a 46% sucrose cushion. Further enrichment of organelles was performed using flotation discontinuous sucrose gradients.

Protein Separation -2D Gels

Proteins in sub cellular compartments were dissolved with a mixture of urea, thiourea, CHAPS, TBP and SB-10 (BioRad ReadyPrep Sequential Extraction Kit, Reagent 3). First-dimension separation (IEF) separation was performed on an Amersham Pharmacia Multiphor II using immobilized pH gradient strips (pI 3-10 linear, 18cm). Second dimension, proteins were separated on a 10% SDS polyacrylamide gel and stained with SYPRO Ruby (BioRad).

Image Analysis

2D SDS PAGE gels were imaged with a Typhoon 8600 Variable Mode Imager (Amersham Pharmacia Biotech). 2D gels were compared and analysed using Amersham Pharmacia ImageMaster image analysis software.

MALDI TOF

Labelled proteins within the gel were excised and the gel slices were washed successively with Tris-HCl, EDTA and mercaptoethanol, ammonium bicarbonate and aceonitrile, isopropanol, and finally digested with trypsin. Peptides were extracted with 1% TFA, dried *in vacuo* and analyzed by Matrix Assisted Laser Desorption Ionisation-Time of Flight (MALDI-TOF). The mass spectra were searched against public protein databases using Protein Prospector Software (Applied Biosystems)

Results and Discussion

The resolving power and reproducibility of 2D SDS PAGE makes it an excellent candidate for monitoring up and down regulation of proteins with respect to cell wall changes. Changes in protein abundances are monitored at various time points in the growth of coleoptiles and during wall regeneration by protoplasts, both of which have a high rate of change with respect to cell wall constituency.

2D SDS PAGE resolves greater than 800 proteins in the Golgi apparatus of barley. The limited size of protein and genomic databases of Barley means that mass fingerprinting of isolated proteins produces few identifications. Protein and peptide sequencing of 2D SDS PAGE proteins is very slow even when mass spectrometry *de novo* sequencing is being used.

To discover wall synthesis proteins, it is necessary to reduce markedly the number of possible protein candidates in individual cellular compartments. In order to do this, we have developed methods to differentially label barley with fluorescent labels at different stages of cell wall development. These methods were developed for a rice cell culture and are currently being applied to barley. Wall characterization is being performed by monosaccharide analysis using gas chromatography-mass spectrometry and wall constituency correlated with protein expression. This is a less than perfect correlation as wall constituency as measured by monosaccharide composition is an average of growth over many hours to days, whereas protein expression is measured in the turnover time of proteins which may be in hours.

Conclusion

A precise definition of the protein profile in regenerating protoplasts will provide information on genes that are being expressed at a time when wall regeneration is the major biochemical activity of a cell. Protein identification is in its infancy and is slow due to the limited coverage available in genomic and proteomic databases. This is expected to increase rapidly as more genomic sequence becomes available. Identification of protein function is still an area of difficulty. To identify protein function requires being able to determine minor changes in cell wall composition than is currently available by monosaccharide analysis and electron microscopy.

Acknowledgments

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