

Proteomics

Finding Function in a Complex World

Complements and interacts directly with genome sequencing programmes

Key methodologies

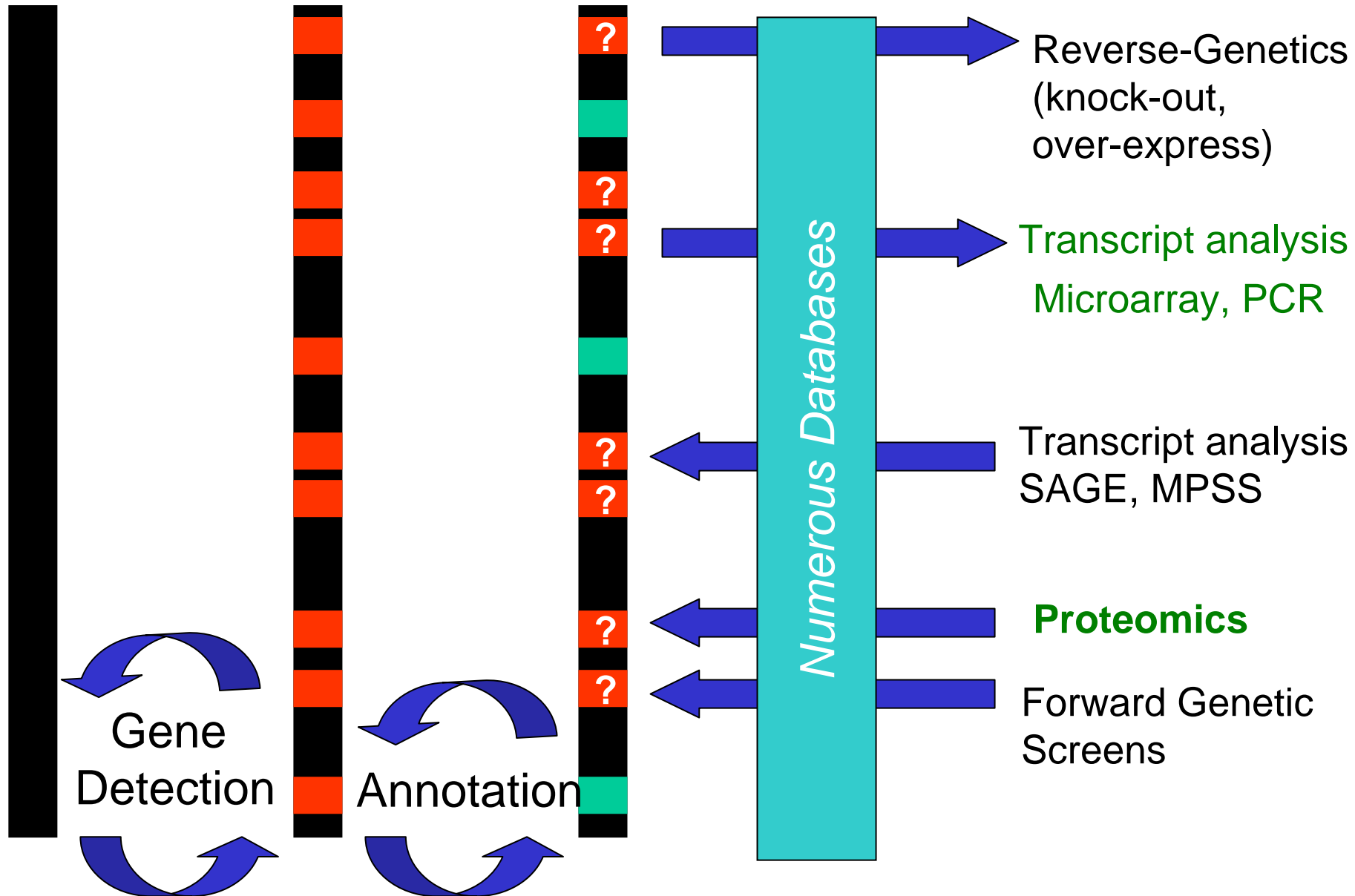
- 2D gel separations of proteins

- image analysis

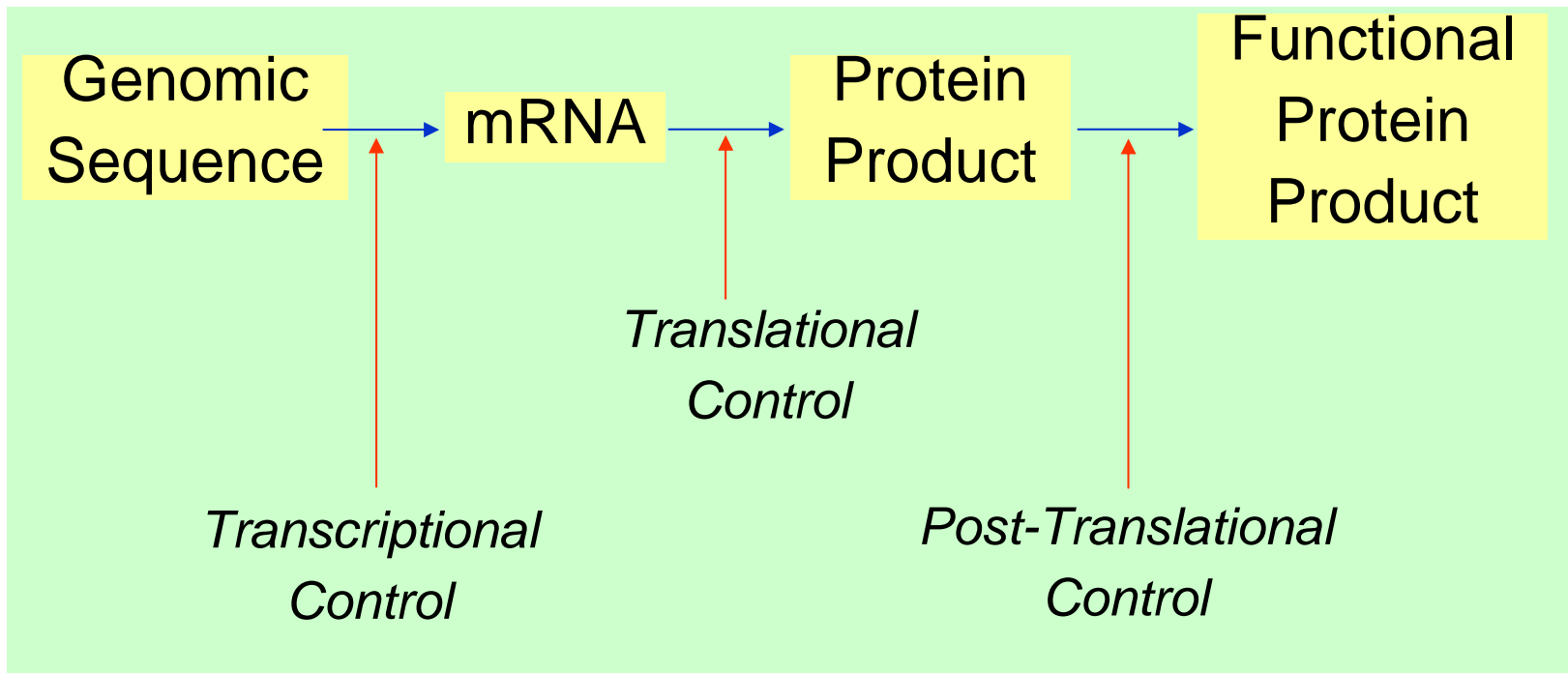
- Mass Spec protein identification using the genome databases

Need to address expression changes at the level of proteins

Functional Genomics: A constantly moving picture of understanding nuclear genomes

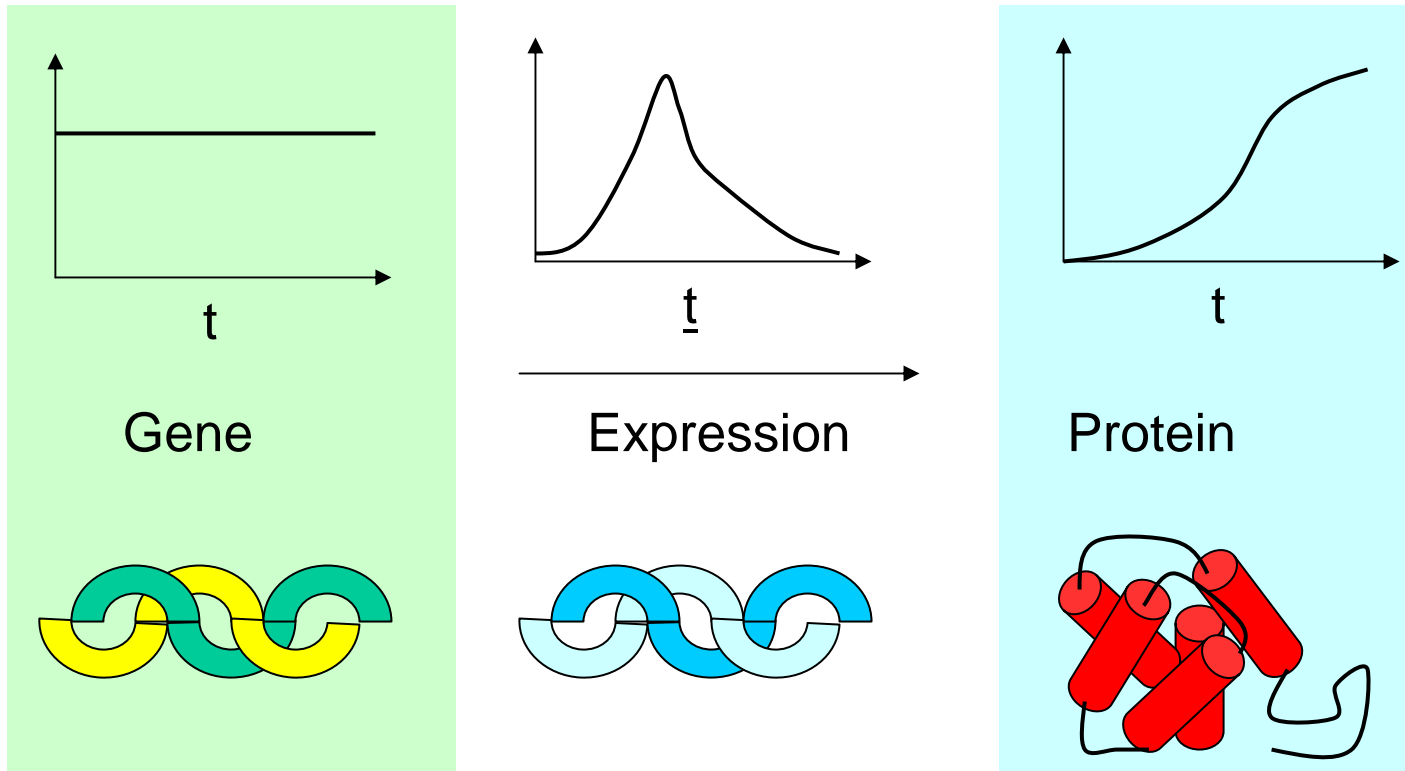


Control of gene expression at different levels



mRNA abundance does not equal expressed protein abundance nor does it indicate the nature of the functional protein product

Temporal Changes in mRNA and protein

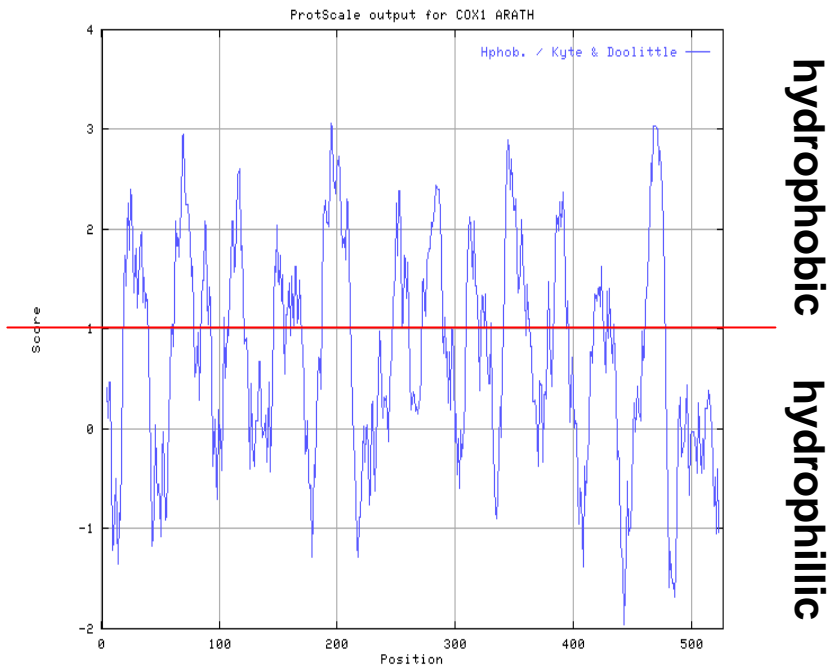


When you measure expression affects what you find

Applications of Proteomics

1. Finding expression, localisation and function for genomic sequences
2. Developing diagnostic markers for disease
3. Identifying proteins responsible for disease states which may be targets for drug engineering
4. Defining molecular elements in signal transduction pathways
5. Identifying protein changes associated with genetic manipulation

1. Probing expression and localisation of unknown proteins



Genome sequencing has identified a large number of predicted protein sequences of unknown function and unknown localisation.

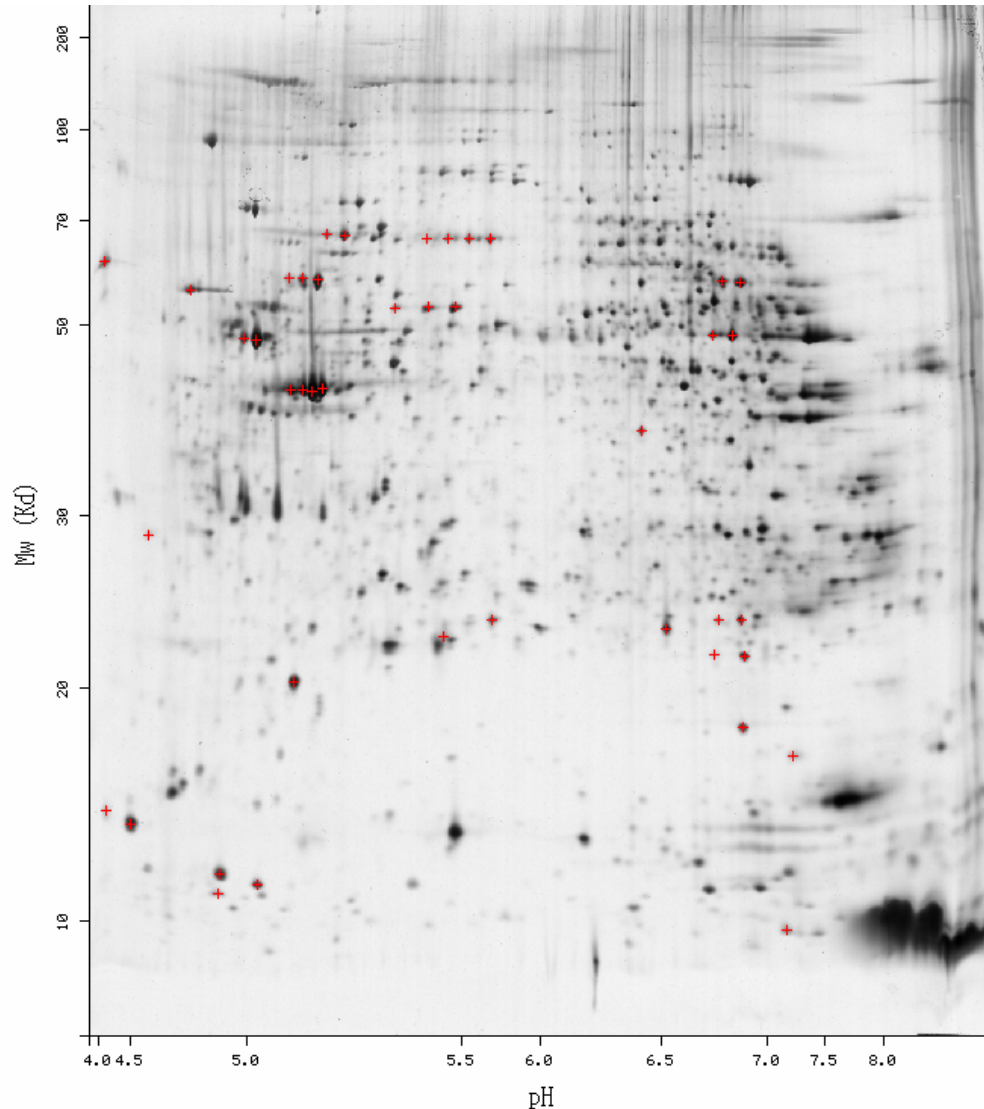
Currently - 40% of protein predicted from genome sequencing cannot be prescribed function on the basis of predicted structure

Proteomic analysis in cells provides

- expression**
- localisation**
- assessment of protein abundance**

**Shevchenko et al.
Proc. Natl Acad. Sci USA
1996
93:14440-14445**

Proteomes of Specific Tissues



<http://expasy.proteome.org.au/ch2d/>

SwissProt 2D Database of Human Kidney Cell Proteins

2789 polypeptides in map

1421 matched to genes

62% known function

Proteomes of Specific Organelles

Compartmentation of the eukaryotic cell
used for analysis of sub-proteomes or organelle-proteomes

1. Nucleus	10%
2. Mitochondria	10%
3. Microsomes (plasma membrane, ER, golgi)	20%
4. Cytosol	70%

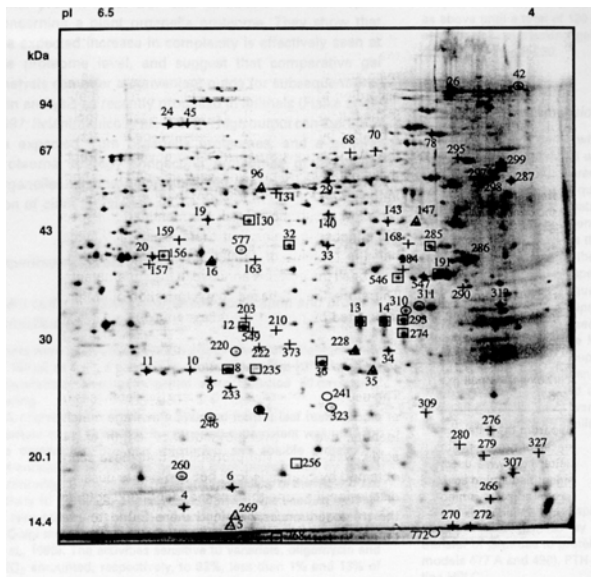
Differential centrifugation (supernatant and pellet)
Gradient centrifugation (density bands)

Arabidopsis thaliana -Plasma Membrane

1997 - 50 proteins known to be located on PM by conventional approaches

1998 - Santoni *et al.* Plant Journal 16:633-641
355 proteins on gels, 50 identified

2003 - Nuhse *et al.* 2003 Mol Cell Proteomics. 2:1234-1243.
Over 200 proteins identified



Known function	21%
EST	50%
no close matches	29%

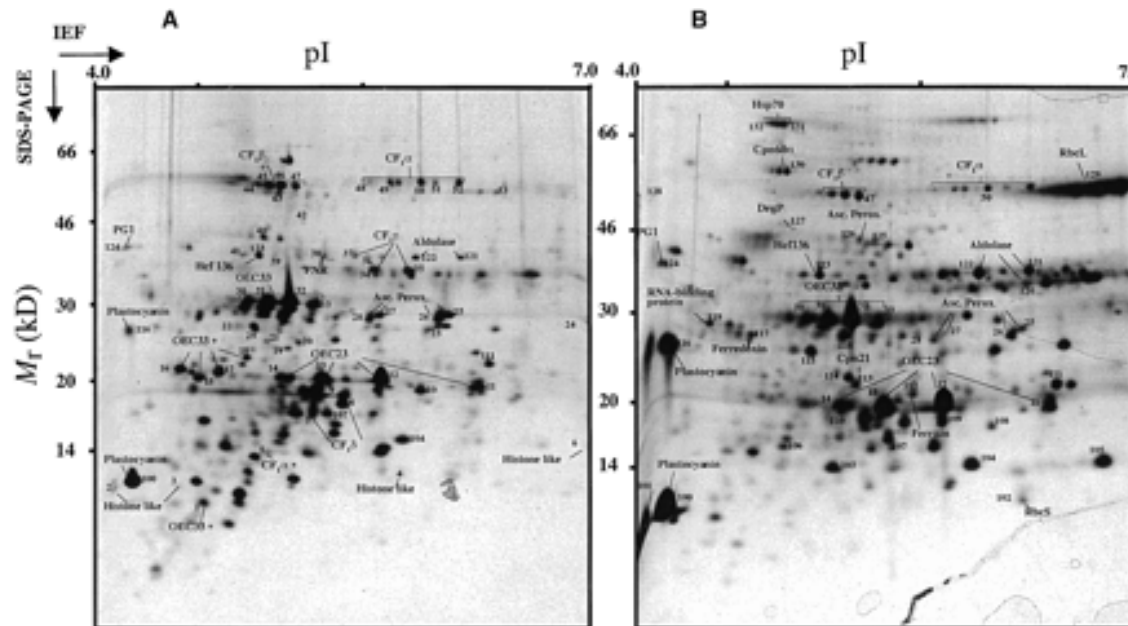
Chloroplasts

Proteomics of the Chloroplast: Systematic Identification and Targeting Analysis

Peltier, Plant Cell 12:319-342 2000

Friso Plant Cell. 2004 16:478-99.

Kleffmann Curr Biol. 2004 14:354-62

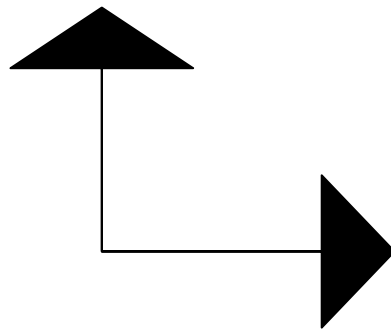
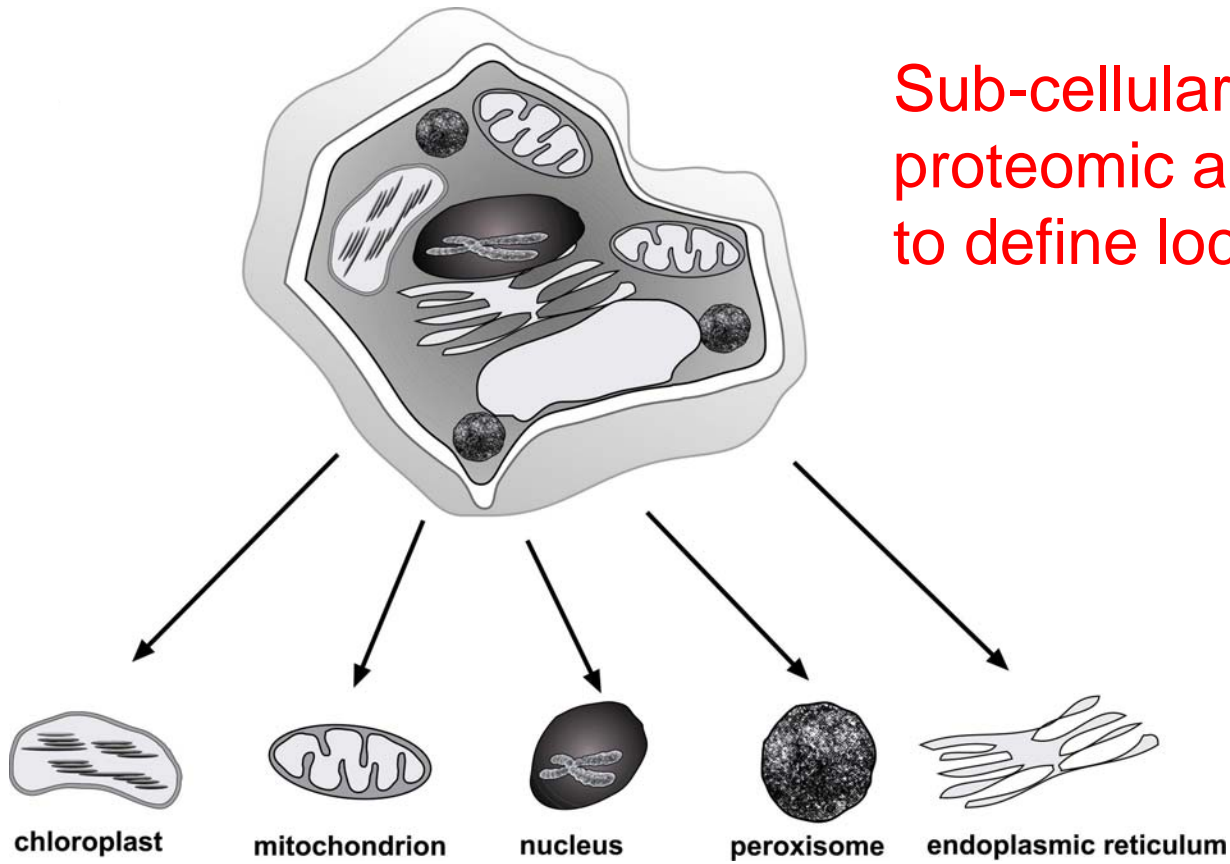


**~1000 proteins on
2D gels**

574 matched to genes

**33% genes with
assigned functions**

Sub-cellular proteomic analysis to define location

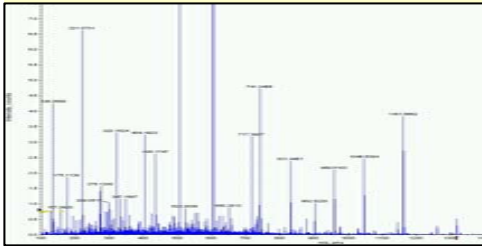


Non-redundant sets of proteins identified in subcellular locations in rice and Arabidopsis.

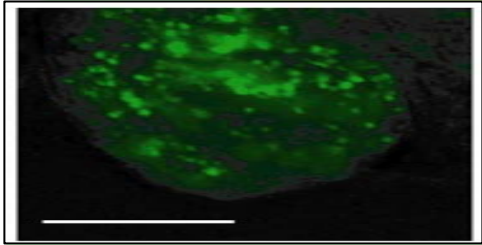
	Arabidopsis	Rice
Chloroplast	574	66
Mitochondrion	409	121
Nucleus	185	-
Peroxisome	35	-
Glyoxysome	19	-
Endoplasmic reticulum	-	-
Plasma membrane	248	90
Golgi	-	44
Tonoplast	-	43
Cell Wall	69	111
Total	1539	475

Rice (Heazlewood *et al.* 2003b; Komatsu *et al.* 2004; Naoki *et al.* 2004), Arabidopsis chloroplast (Ferro *et al.* 2003; Friso *et al.* 2004; Froehlich *et al.* 2003; Peltier *et al.* 2002; Schubert *et al.* 2002), (Heazlewood *et al.* 2004) and references therein for the mitochondrion, nucleus (Bae *et al.* 2003; Calikowski *et al.* 2003), plasma membrane (Borner *et al.* 2003; Elortza *et al.* 2003; Prime *et al.* 2000; Santoni *et al.* 2003, Nuhse *et al.* 2003), cell wall (Chivasa *et al.* 2002), peroxisome/glyoxysome (Fukao *et al.* 2003; Fukao *et al.* 2002).

Combining GFP, Mass Spec and Predictions



38 papers on major subcellular proteomes by MS
2871 claims for subcellular location
~2400 non-redundant At proteins localised



920 papers mentioning (G,Y,C,R)FP and Arabidopsis
425 with subcellular localisations
~1100 non-redundant At proteins localised



1820 entries for Arabidopsis
with subcellular location annotation in comments



30,481 proteome predictions for Arabidopsis
with 7 target prediction programs



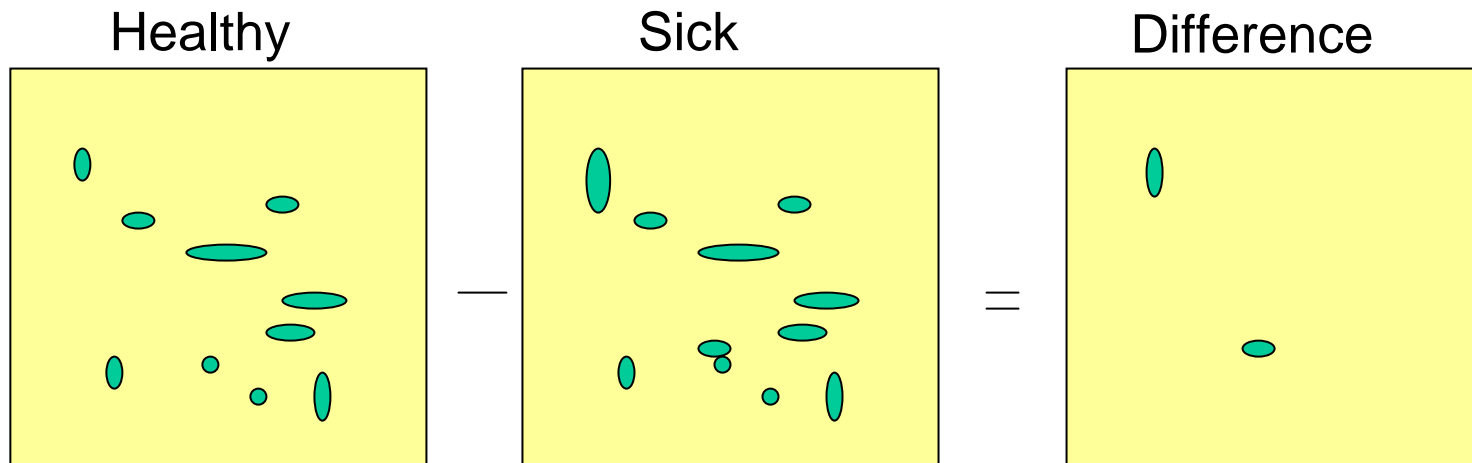
1037 IDA – Direct Assay
with sub-cellular location evidence

2. Diagnosis of Disease

- Large range of single measurement diagnostic tests are available
- Need for single tests that provide data on complex associations

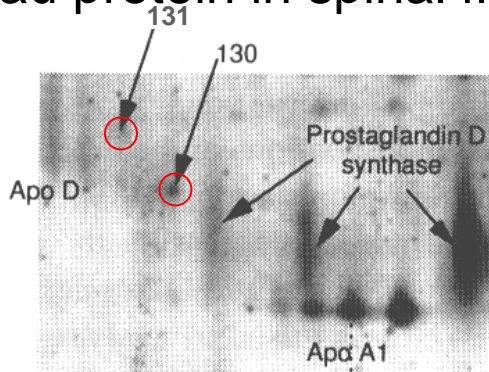
Applications currently in use:

- identifying the origin of body fluid samples
- analyzing the clonality of immunoglobulins
- monitoring protein expression in nutritional disorders
- discovering new disease markers/patterns

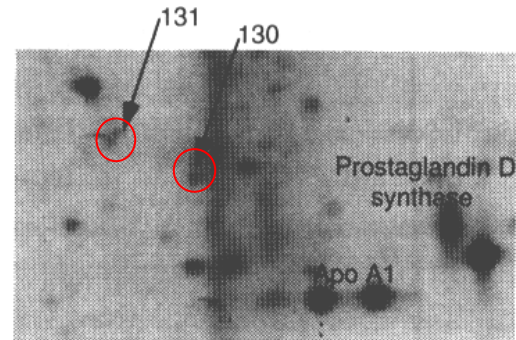


Proteome markers for Creutzfeldt-Jakob disease (human form of Mad Cows' disease)

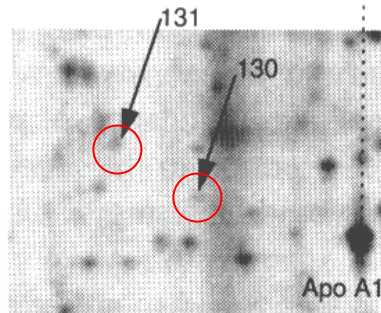
Prior to 1996 - no premortem biochemical diagnostic test for CJD
Brain Tau protein in spinal fluid - a new disease marker (1996)



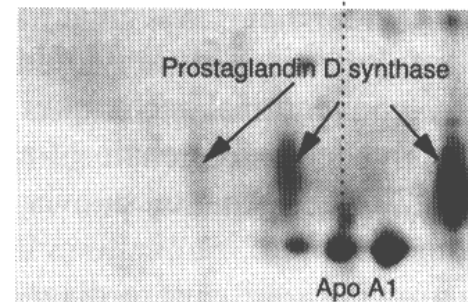
CJD spinal fluid



Astrocytes + CJD spinal fluid



Brain Astrocytes



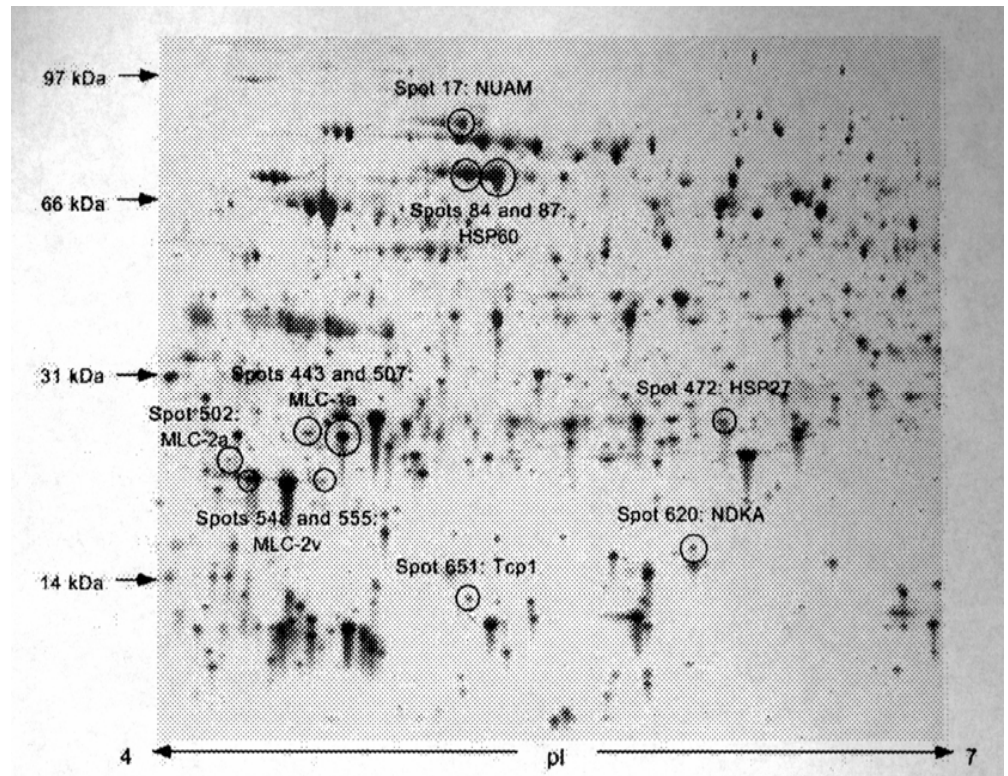
Normal spinal fluid

3. Identification of proteins associated with Heart Disease

Cardiac myocyte hypertrophy
(excessive growth of heart muscle cells)

This physiology is linked to congestive heart failure

What changes in expression occur during this transition of myocytes?



Arnott et al 1998: Analytical Biochemistry 258:1-18

Dunn 2000: Drug Discovery Today 5:76-84

Prentice & Webster 2004: Trends in Cardiovascular Medicine 14:282-288

Chaperonin/signaling/stress proteins (2)



40% decrease in Chaperonin cofactor a, HSP27
(changes in cytoskeletal organisation of tubulin)

40% decrease in nucleoside diphosphate kinase
(regulates NTP levels/interaction with G protein signaling)



Myosin light chains (5)

40-60% increase

(reversion to embryonic programme of gene expression, myosin required for contraction of muscle cells)



Mitochondrial and energy metabolism proteins (3)

40% decrease HSP60, complex I subunit

(decreased energy metabolism)



Ubiquitination pathway proteins (1)

700% increase ubiquitin carboxyl-terminal hydrolase

(increase or inappropriate ubiquitination and breakdown of proteins)

Fast tracking New Drug Discovery ?

Pharmaceutical Industry has large arrays of 'potential drugs'
-don't know what they do or what condition they might help

Need for matching up drugs with disease

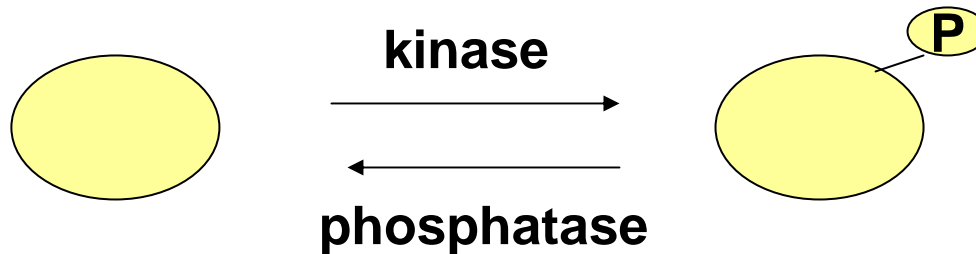
- Screen all the drugs for the changes they invoke
- Screen all the diseases for the changes they invoke

Match drugs that decrease a given protein or set of proteins
with a disease that causes them to rise.

Do some clinical tests/trials etc.

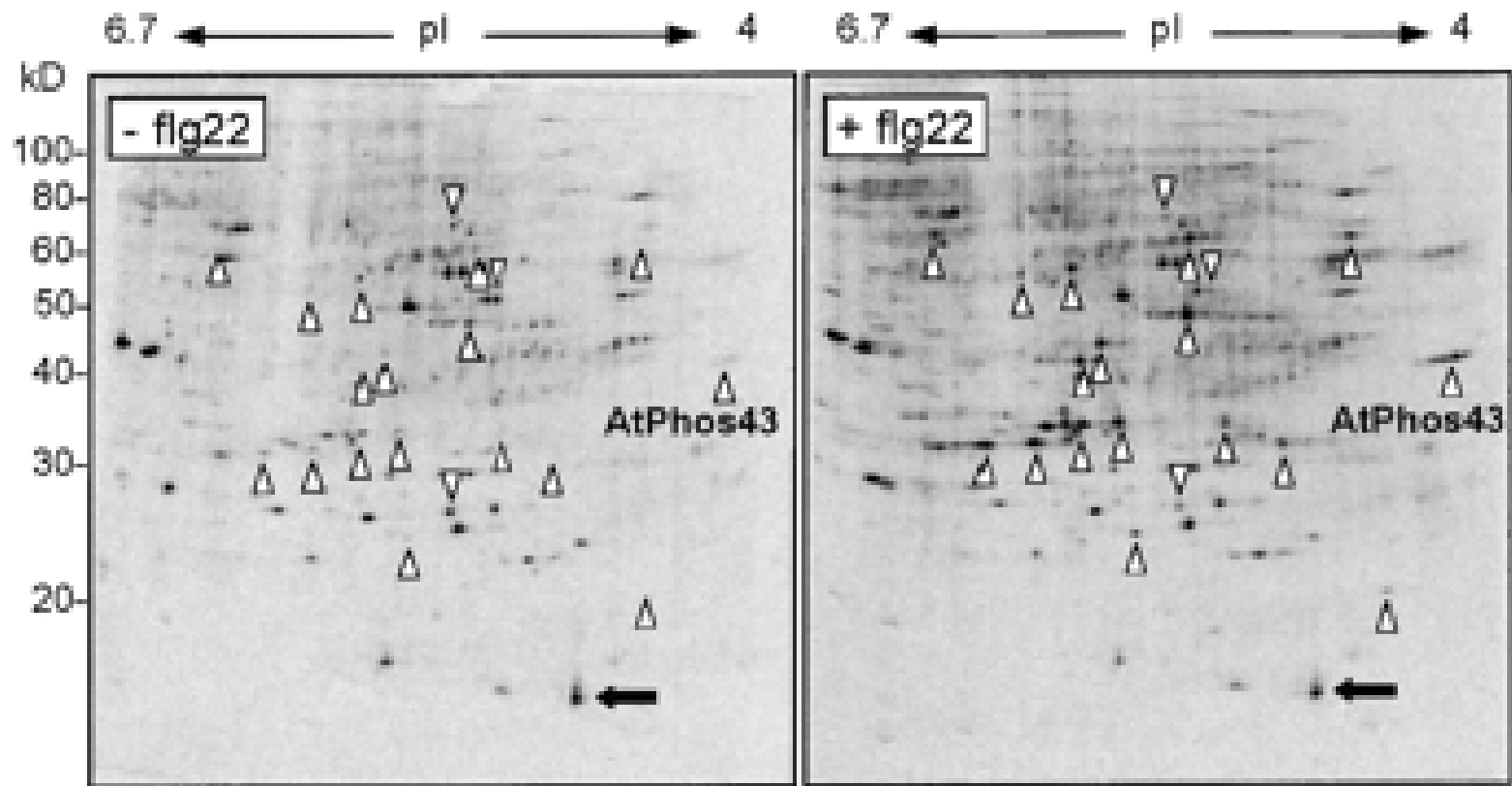
4. Defining molecular elements in signal transduction pathways

Many of the rapid responses of cells to environmental changes are not at the level of transcription and translation (these are too slow), but operate through reversible post-translational processes such as phosphorylation.

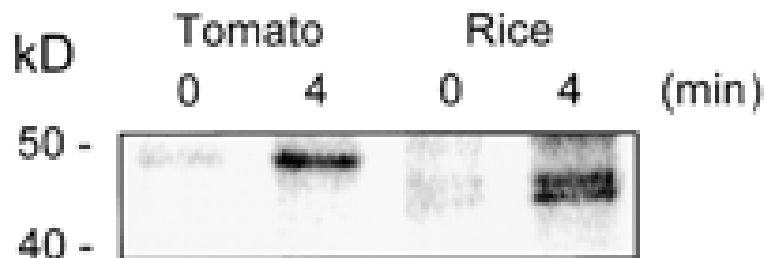


For example, plants respond to invasion by bacteria and fungi by receptors that recognise proteins/compounds from these pathogens and then begin kinase cascades to ready the plant for an imminent attack.

³²P label incorporation in response to fungal elicitor (Arabidopsis)



Rapid response of homologs in crop plants



Peck et al 2001,
The Plant Cell 13:1467-1475

5. Searching for holistic changes caused by a single genetic manipulation

- Changing expression of a single gene may causes changes in expression of other genes and thus alters the total gene expression pattern
- A key concern in genetic manipulation

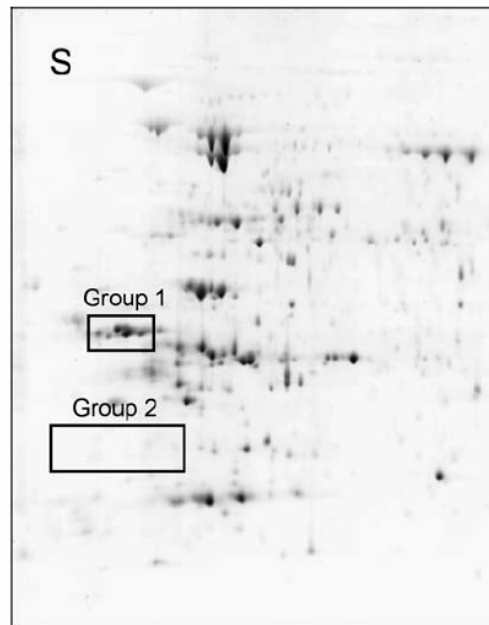
FEBS Lett. 2004 576:477-80.

Expression proteomics identifies biochemical adaptations and defense responses in transgenic plants with perturbed polyamine metabolism.

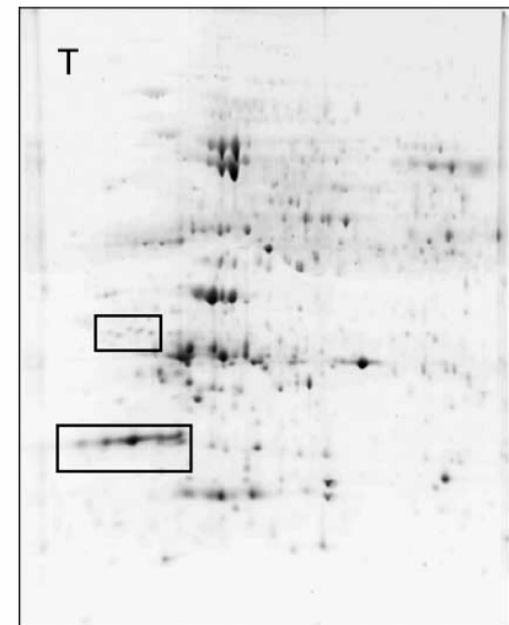
Franceschetti M, Perry B, Thompson B, Hanfrey C, Michael AJ.

**S-adenosylmethionine
decarboxylase
overexpressed**

**LESS POLYAMINES
PRODUCED**



Control



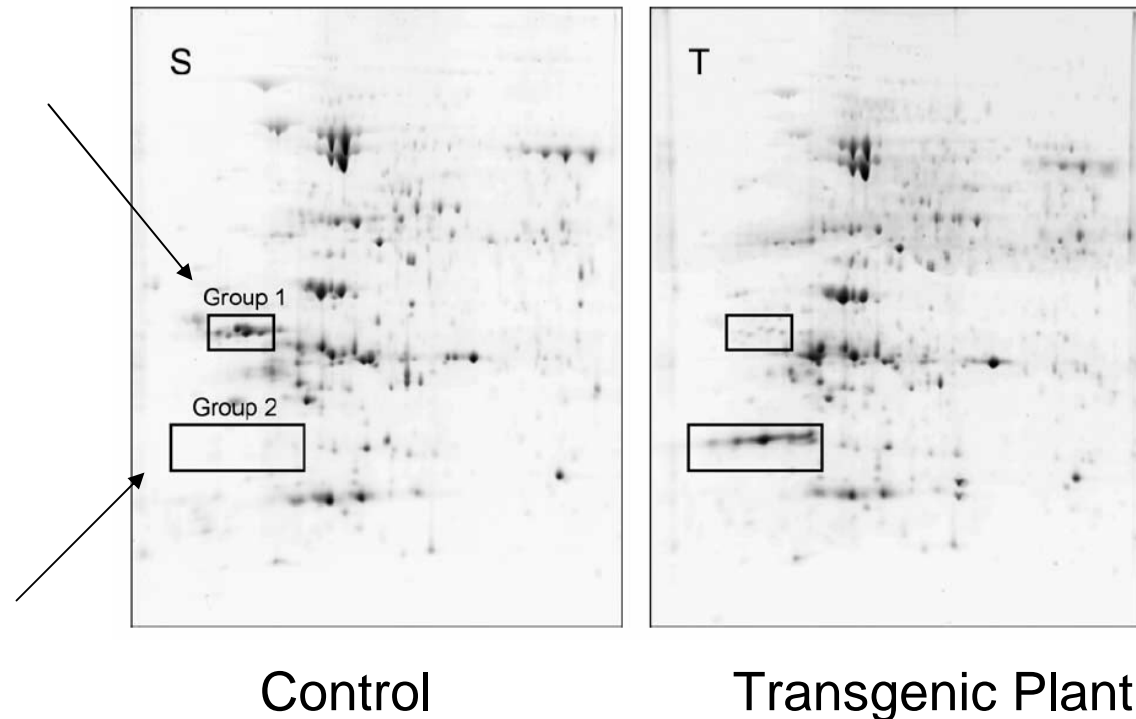
Transgenic Plant

“stunted growth, wrinkled leaves and inhibition of cell expansion”

Decreasing proteins

(Group 1) were identified as isoforms of chloroplast ribonucleoproteins, known to be involved in chloroplast mRNA stability, processing and translation.

Another group of eight proteins **strongly induced** (Group 2) were identified as multiple, isoforms of the defense protein PR-1.



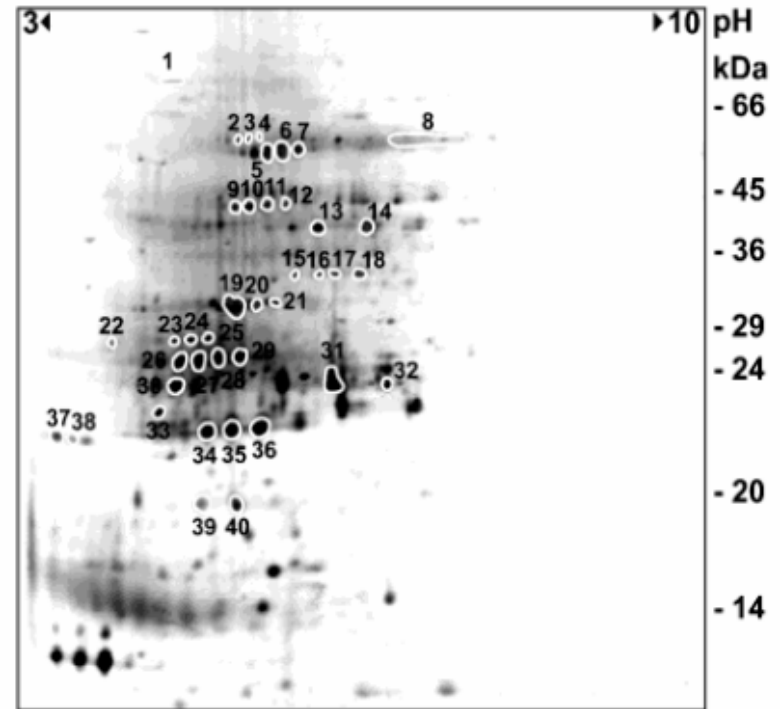
Proteomics. 2004 4:193-200.

Proteomics as a tool to improve investigation of substantial equivalence in genetically modified organisms: the case of a virus-resistant tomato.

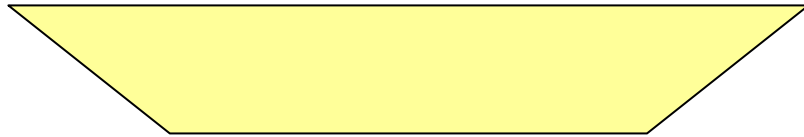
Corpillo D, Gardini G, Vaira AM, Basso M, Aime S, Accotto GP, Fasano M.

Compared protein expression of two types of tomato plants, having the same genetic background, except for a virus resistance trait introduced by genetic engineering.

When proteins extracted from seedlings of the two types were analyzed by two-dimensional electrophoresis, no significant differences, either qualitative or quantitative, were detected, indicating that in this case the expression of major proteins was unmodified by the genetic manipulation.



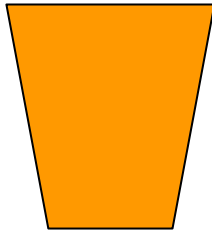
Proteomics Applied



Finding Function/Localisation
of Predicted Gene Products



Documenting gene product expression patterns
associated with modified state



Identifying candidates
-for drug development
-for genetic manipulation



Testing effect of Drug/Genetic manipulation
on gene product expression patterns

So what is happening at UWA?

- 1998- 3-4 laboratories began 2D gel array and image analysis work in relation to their research.**
- 1999 - Lotteries funded DNA arrayers and readers by Affymetrix**
- 1999 - UWA administration funded a bid from Science, Medicine, Agriculture and Engineering for a 'Genomics Centre' - initially \$ 1 M**
- 2000 - The Australian Research Council funded purchase of a state-of-the-art mass spectrometer for protein analysis - ~ \$ 1 M**
- 2001 –Funding for proteomics robotics funded (\$0.5M)**
- 2002 – Improvements to mass spectrometer funded (\$0.2M)**
- 2004- Major funding for Transcriptomics and Proteomics (\$5M)**
- 2005- ARC Centre of Excellence in Plant Energy Biology (\$12.5M)**
- 2006- NCRIS funding for metabolomics in WA (\$2M)**