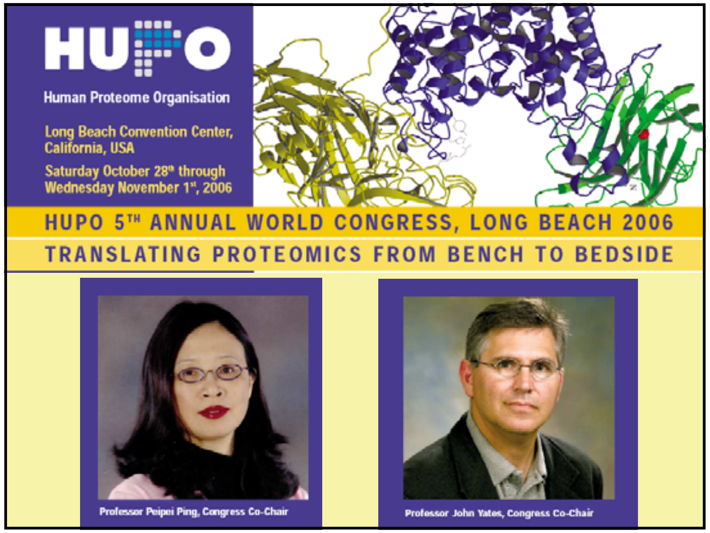
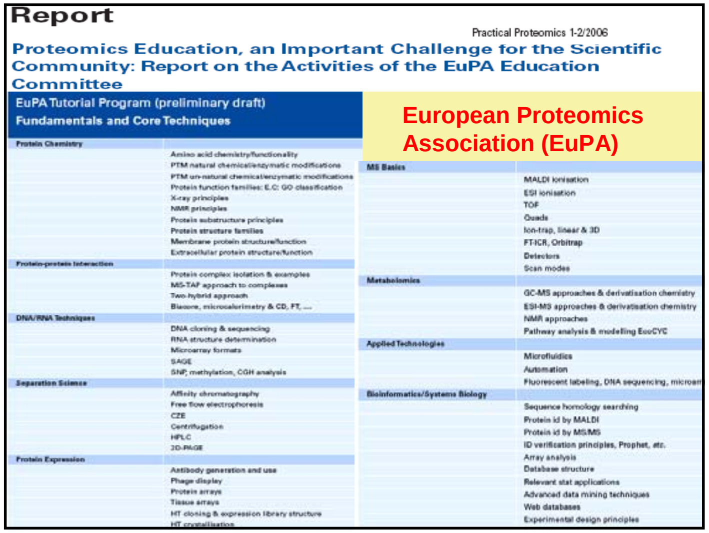
Mass Spec and MicroArrays

Applications in Proteomics and Systems Biology

2007 HUPO Seoul
HUPO 6th ANNUAL WORLD CONGRESS, SEOUL 2007
HUPO
Oct.6th to Oct.10th, 2007 COEX, Seoul, Korea
Proteomics: From Technology Development to Biomarker Applications
Co-sponsored by HUPO, AOHUPO and KHUPO

CH370 - Hackert


HUPO
Human Proteome Organisation
Long Beach Convention Center, California, USA
Saturday October 28th through Wednesday November 1st, 2006
HUPO 5TH ANNUAL WORLD CONGRESS, LONG BEACH 2006
TRANSLATING PROTEOMICS FROM BENCH TO BEDSIDE
Professor Peipei Ping, Congress Co-Chair
Professor John Yates, Congress Co-Chair



Report

Practical Proteomics 1-2/2006

Proteomics Education, an Important Challenge for the Scientific Community: Report on the Activities of the EuPA Education Committee

European Proteomics Association (EuPA)

EuPA Tutorial Program (preliminary draft)
Fundamentals and Core Techniques

Protein Chemistry	
	Amino acid chemistry/functionality
	PTM natural chemical/enzymatic modifications
	PTM un-natural chemical/enzymatic modifications
	Protein function families: E.C: GO classification
	X-ray principles
	NMR principles
	Protein substructure principles
	Protein structure families
	Membrane protein structure/function
	Extracellular protein structure/function
Protein-protein interaction	
	Protein complex isolation & examples
	MS-TAP approach to complexes
	Two-hybrid approach
	Biacore, microcalorimetry & CD, FT, ...
DNA/RNA Techniques	
	DNA cloning & sequencing
	RNA structure determination
	Microarray formats
	SAGE
	SNP, methylation, CGH analysis
Separation Science	
	Affinity chromatography
	Free flow electrophoresis
	CZE
	Centrifugation
	HPLC
	2D-PAGE
Protein Expression	
	Antibody generation and use
	Phage display
	Protein arrays
	Tissue arrays
	HT cloning & expression library structure
	HT crystallisation
MS Basics	
	MALDI ionisation
	ESI ionisation
	TOF
	Quads
	Ion-trap, linear & 3D
	FT-ICR, Orbitrap
	Detectors
	Scan modes
Metabolomics	
	GC-MS approaches & derivatisation chemistry
	ESI-MS approaches & derivatisation chemistry
	NMR approaches
	Pathway analysis & modelling EcoCYC
Applied Technologies	
	Microfluidics
	Automation
	Fluorescent labeling, DNA sequencing, microarr
Bioinformatics/Systems Biology	
	Sequence homology searching
	Protein id by MALDI
	Protein id by MS/MS
	ID verification principles, Prophet, etc.
	Array analysis
	Database structure
	Relevant stat applications
	Advanced data mining techniques
	Web databases
	Experimental design principles

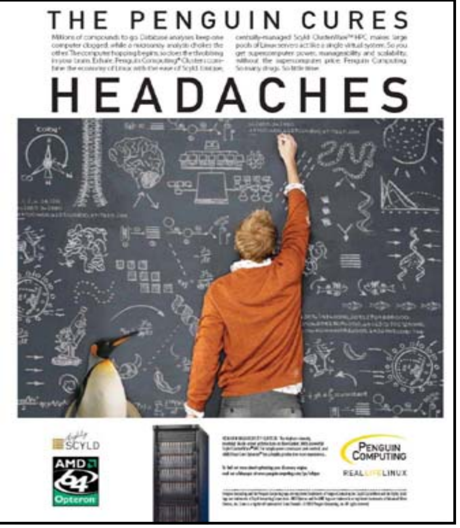
Genomics

Proteomics

Interactomics

Systems Biology –

None of these fields of research would be possible without **Bioinformatics,** which would not be possible with lots of **computing power!**



Mass Spec and MicroArrays / Applications

Genome – the genome of an organism is its whole hereditary information encoded in its DNA (or, RNA for some viruses) and includes both the coding (genes) and non-coding sequences of the DNA.

Proteome – Proteomics is often considered the next step in the study of biological systems, after genomics. It is much more complicated than genomics, mostly because while an organism's genome is rather constant, a proteome differs from cell to cell and constantly changes through its biochemical interactions with the genome and the environment.

Interactome – whole set of molecular interactions in cells, in the context of proteomics, it refers to protein-protein interaction network(PPI), or protein network (PN).

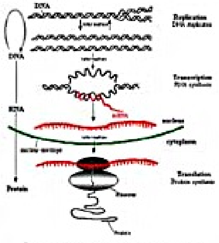
Systems Biology - seeks to understand how biological systems function. By studying the relationships and interactions between various parts of a biological system (e.g. metabolic pathways, organelles, cells, physiological systems, organisms etc.), it is hoped that eventually a model of the whole system can be developed.

The Proteome

All an organism's cells carry the same Genome, and it is Static. Genomes do not describe function. They are a parts list.

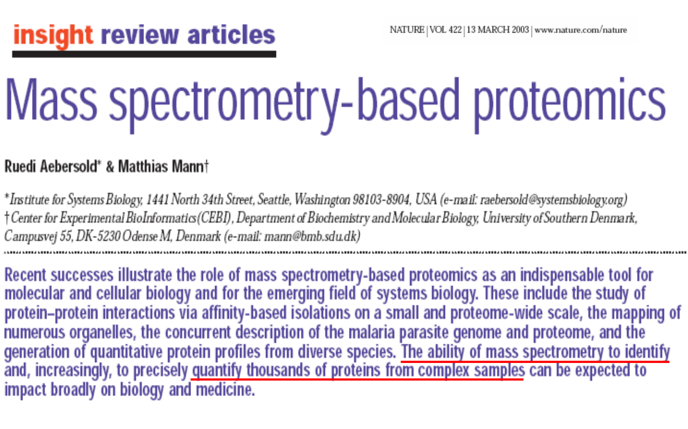
Different cells express different proteins. The type and quantity of this expression changes.

The Proteome is Dynamic. It is the total of all proteins expressed by a particular *cell* at a given *time*, under specific *conditions*.



The Central Dogma of Molecular Biology

A Proteome cannot be studied the way a Genome is sequenced. There has to be a specific biological question behind an experiment. The questions may be either very broad or strictly defined.



insight review articles

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Mass spectrometry-based proteomics

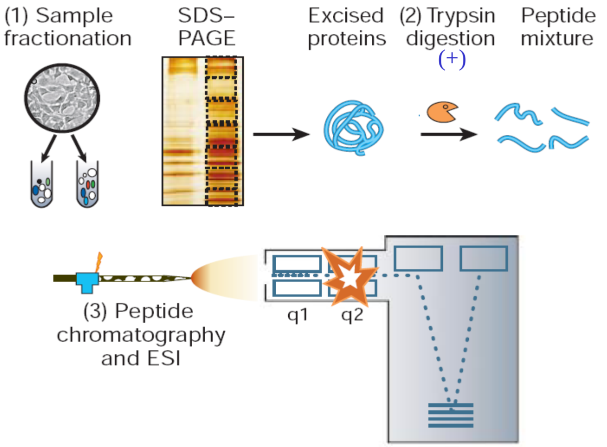
Ruedi Aebersold* & Matthias Mann†

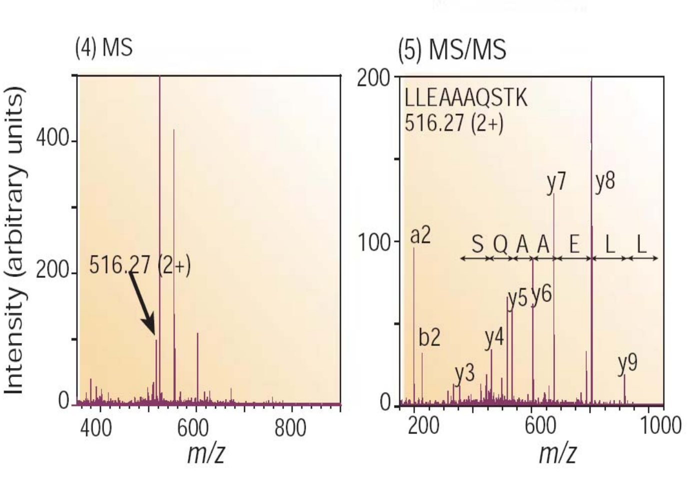
*Institute for Systems Biology, 1441 North 34th Street, Seattle, Washington 98103-8904, USA (e-mail: raebersold@systemsbiology.org)
†Center for Experimental BioInformatics (CEBI), Department of Biochemistry and Molecular Biology, University of Southern Denmark, Campusvej 55, DK-5230 Odense M, Denmark (e-mail: mann@bmb.sdu.dk)

Recent successes illustrate the role of mass spectrometry-based proteomics as an indispensable tool for molecular and cellular biology and for the emerging field of systems biology. These include the study of protein–protein interactions via affinity-based isolations on a small and proteome-wide scale, the mapping of numerous organelles, the concurrent description of the malaria parasite genome and proteome, and the generation of quantitative protein profiles from diverse species. The ability of mass spectrometry to identify and, increasingly, to precisely quantify thousands of proteins from complex samples can be expected to impact broadly on biology and medicine.

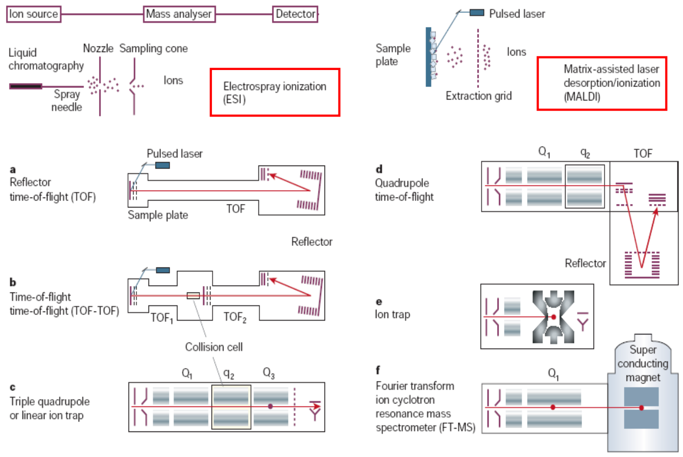
Note: HT Proteomics is restricted to those species where a sequence database exists!

Generic Mass Spectrometry-based Proteomics

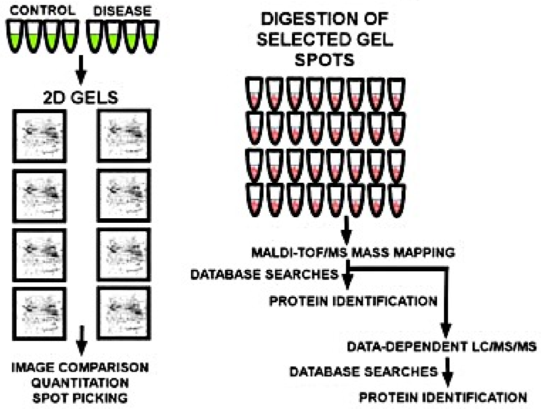




Common MSs Used in Proteomics Research


Ion source
Mass analyser
Detector
Liquid chromatography
Nozzle
Sampling cone
Ions
Spray needle
Electrospray ionization (ESI)
Pulsed laser
Sample plate
Ions
Extraction grid
Matrix-assisted laser desorption/ionization (MALDI)
a
Reflector time-of-flight (TOF)
Pulsed laser
Sample plate
TOF
Reflector
b
Time-of-flight time-of-flight (TOF-TOF)
TOF1
TOF2
Collision cell
c
Triple quadrupole or linear ion trap
Q1
q2
Q3
d
Quadrupole time-of-flight
Q1
q2
TOF
Reflector
e
Ion trap
f
Fourier transform ion cyclotron resonance mass spectrometer (FT-MS)
Q1
Super conducting magnet

Differential Expression Proteomics



Two Dimensional Gel Electrophoresis

Isoelectric focusing is performed on precast gel strips using commercial instruments. Many pH ranges are available. Multiple strips can be run in parallel.

An immobilized pH gradient is created in a polyacrylamide gel strip by incorporating a gradient of acidic and basic buffering groups when the gel is cast.

Resolution is determined by the slope of the pH gradient and the field strength.

Loading capacity depends on gel size and thickness.

In 2D IEF/PAGE, the gel strip from IEF is loaded into a single large well.

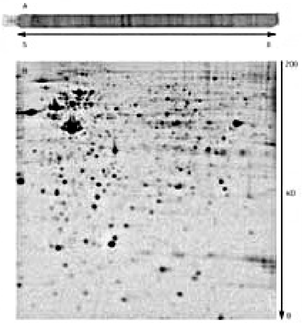


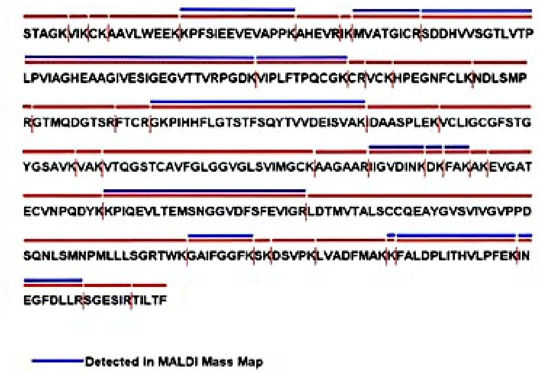
Fig. 1. Principle of 2-D electrophoresis. A, pre-B lymphoma cell extract (1 mg) was separated by IEF on a ReadyStrip pH 5–8 IPG strip, and stained with Bio-Safe™ Coomassie stain. B, Equilibrated strip was run in the second dimension by SDS-PAGE (12% acrylamide). The gel was stained with Coomassie Blue.

Figure from BioRad Product Literature

With the new genomic data bases of model species, such as *Escherichia coli, Saccharomyces cerevisae,* mouse, and human, the sequences of many/most proteins of biological interest will in principle be known, and the problem of characterizing a protein primary structure will be reduced to identifying it in the data base.

Within the past few years research groups have demonstrated how MS can be used for identification of proteins in sequence data bases. One approach is to **cleave the protein with a sequence-specific proteolytic enzyme**, **measure molecular weight** values for the resulting peptide mixture by mass spectrometry, and **search a sequence data base for proteins that should yield these values**. **Search algorithms** can utilize low resolution tandem mass spectra of selected peptides (<3 kDa) from the protein degradation. Yates and coworkers compared the MS/MS sequence data to the sequences predicted for each of the peptides that would be generated from each protein in the data base. **In the PEPTIDESEARCH sequence tag approach of Mann and Wilm, a partial sequence of 2–3 amino acids is assigned from the fragment mass differences in the MS/MS spectrum.** This partial sequence and its mass distance from each end of the peptide (based on the masses of the fragment and molecular ions) are used for the data base search. Often, **a single sequence tag retrieved only the correct protein from the data base**.

Tryptic Digest of ADH: Expected Peptides vs. Those Detected



STAGK|VIK|CK|AAVLWEEK|KPFSIEEVEVAPPK|AHEVR|IK|MVATGICR|SDDHVVSGTLVTP

LPVIAGHEAAGIVESIGEGVTTVRPGDK|VIPLFTPQCGK|CR|VCK|HPEGNFCLK|NDLSMP

R|GTMQDGTSR|FTCR|GKPIHHFLGTSTFSQYTVVDEISVAK|IDAASPLEK|VCLIGCGFSTG

YGSAVK|VAK|VTQGSTCAVFGLGGVGLSVIMGCK|AAGAAR|IIGVDINK|DK|FAK|AK|EVGAT

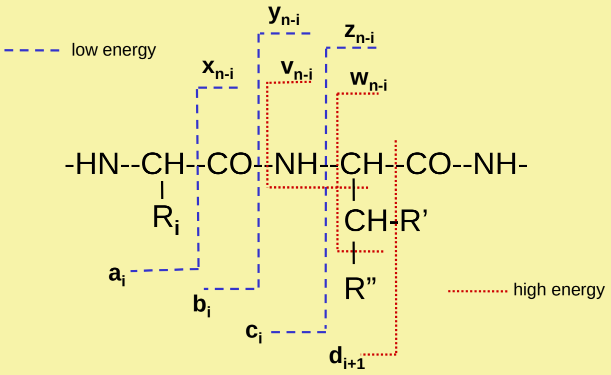
ECVNPQDYK|KPIQEVLTEMSNGGVDFSFEVIGR|LDTMVTALSCCQEAYGVSVIVGVPPD

SQNLSMNPMLLLSGRTWK|GAIFGGFK|SK|DSVPK|LVADFMAK|K|FALDPLITHVLPFEK|IN

EGFDLLR|SGESIR|TILTF

Detected in MALDI Mass Map

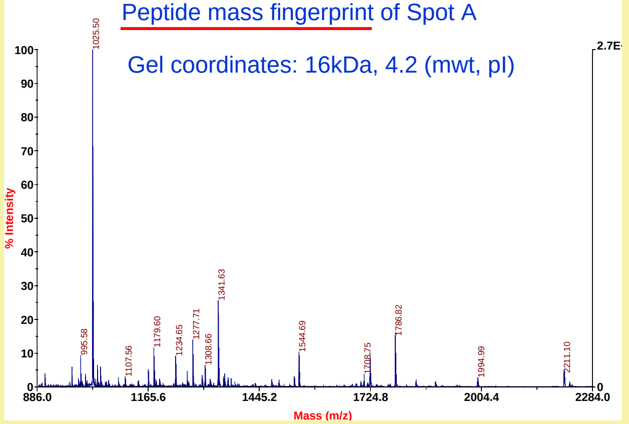
Cleavages Observed in MS/MS of Peptides

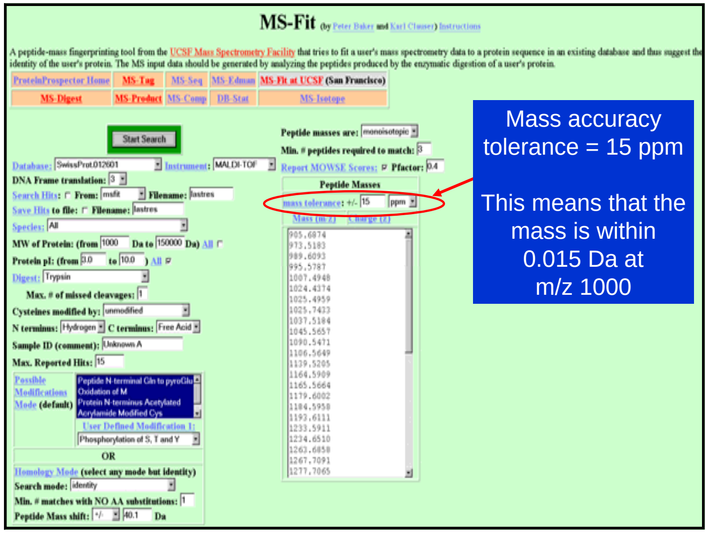


CID (Collision InDuced) Spectra – adds **sequence data** to **mass mapping** for improved database identification!

Peptide mass fingerprint of Spot A

Gel coordinates: 16kDa, 4.2 (mwt, pI)





MS-Fit

(by Peter Baker and Karl Clauser) Instructions

A peptide-mass fingerprinting tool from the UCSF Mass Spectrometry Facility that tries to fit a user's mass spectrometry data to a protein sequence in an existing database and thus suggest the identity of the user's protein. The MS input data should be generated by analyzing the peptides produced by the enzymatic digestion of a user's protein.

ProteinProspector Home	MS-Tag	MS-Seq	MS-Edman	MS-Fit at UCSF (San Francisco)
MS-Digest	MS-Product	MS-Comp	DB-Stat	MS-Isotope

Start Search

Database: SwissProt.012601 Instrument: MALDI-TOF
DNA Frame translation: 3
Search Hits: ☐ From: msfit Filename: lastres
Save Hits to file: ☐ Filename: lastres
Species: All
MW of Protein: (from 1000 Da to 150000 Da) All ☐
Protein pI: (from 3.0 to 10.0) All ☑
Digest: Trypsin
Max. # of missed cleavages: 1
Cysteines modified by: unmodified
N terminus: Hydrogen C terminus: Free Acid
Sample ID (comment): Unknown A
Max. Reported Hits: 15

Possible Modifications Mode (default): Peptide N-terminal Gln to pyroGlu / Oxidation of M / Protein N-terminus Acetylated / Acrylamide Modified Cys
User Defined Modification 1: Phosphorylation of S, T and Y

OR

Homology Mode (select any mode but identity)
Search mode: identity
Min. # matches with NO AA substitutions: 1
Peptide Mass shift: +/- 40.1 Da

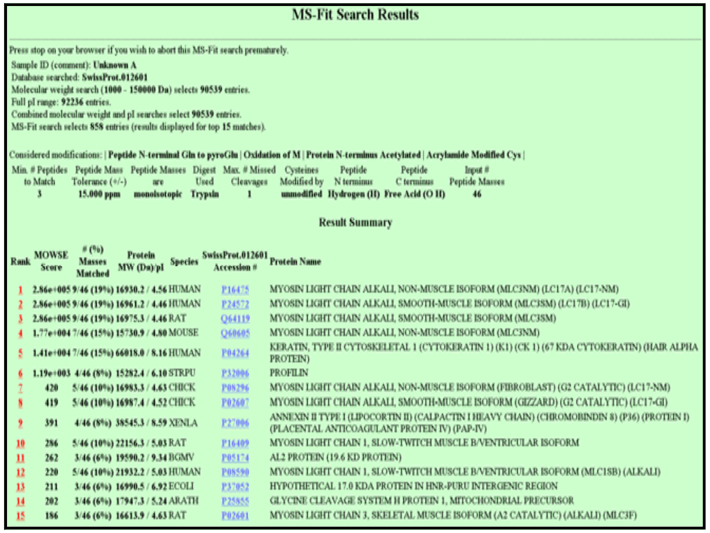
Peptide masses are: monoisotopic
Min. # peptides required to match: 3
Report MOWSE Scores: ☑ Pfactor: 0.4

Peptide Masses
mass tolerance: +/- 15 ppm
Mass (m/z) Charge (z)

```
905.6874
973.5183
989.6093
995.5787
1007.4948
1024.4374
1025.4959
1025.7433
1037.5184
1045.5657
1090.5471
1106.5649
1139.5205
1164.5909
1165.5664
1179.6002
1184.5958
1193.6111
1233.5911
1234.6510
1263.6858
1267.7091
1277.7065
```

Mass accuracy tolerance = 15 ppm

This means that the mass is within 0.015 Da at m/z 1000



MS-Fit Search Results

Press stop on your browser if you wish to abort this MS-Fit search prematurely.

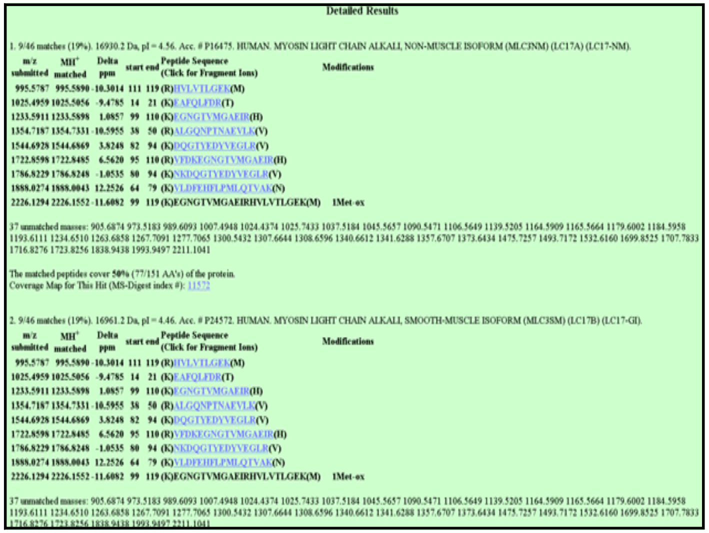
Sample ID (comment): **Unknown A**
Database searched: **SwissProt.012601**
Molecular weight search (**1000 - 150000 Da**) selects **90539** entries.
Full pI range: **92236** entries.
Combined molecular weight and pI searches select **90539** entries.
MS-Fit search selects **858** entries (results displayed for top **15** matches).

Considered modifications: | **Peptide N-terminal Gln to pyroGlu** | **Oxidation of M** | **Protein N-terminus Acetylated** | **Acrylamide Modified Cys** |

Min. # Peptides to Match	Peptide Mass Tolerance (+/-)	Peptide Masses are	Digest Used	Max. # Missed Cleavages	Cysteines Modified by	Peptide N terminus	Peptide C terminus	Input # Peptide Masses
3	15.000 ppm	monoisotopic	Trypsin	1	unmodified	Hydrogen (H)	Free Acid (O H)	46

Result Summary

Rank	MOWSE Score	# (%) Masses Matched	Protein MW (Da)/pI	Species	SwissProt.012601 Accession #	Protein Name
1	2.86e+005	9/46 (19%)	16930.2 / 4.56	HUMAN	P16475	MYOSIN LIGHT CHAIN ALKALI, NON-MUSCLE ISOFORM (MLC3NM) (LC17A) (LC17-NM)
2	2.86e+005	9/46 (19%)	16961.2 / 4.46	HUMAN	P24572	MYOSIN LIGHT CHAIN ALKALI, SMOOTH-MUSCLE ISOFORM (MLC3SM) (LC17B) (LC17-GI)
3	2.86e+005	9/46 (19%)	16975.3 / 4.46	RAT	Q64119	MYOSIN LIGHT CHAIN ALKALI, SMOOTH-MUSCLE ISOFORM (MLC3SM)
4	1.77e+004	7/46 (15%)	15730.9 / 4.80	MOUSE	Q60605	MYOSIN LIGHT CHAIN ALKALI, NON-MUSCLE ISOFORM (MLC3NM)
5	1.41e+004	7/46 (15%)	66018.0 / 8.16	HUMAN	P04264	KERATIN, TYPE II CYTOSKELETAL 1 (CYTOKERATIN 1) (K1) (CK 1) (67 KDA CYTOKERATIN) (HAIR ALPHA PROTEIN)
6	1.19e+003	4/46 (8%)	15282.4 / 6.10	STRPU	P32006	PROFILIN
7	420	5/46 (10%)	16983.3 / 4.63	CHICK	P08296	MYOSIN LIGHT CHAIN ALKALI, NON-MUSCLE ISOFORM (FIBROBLAST) (G2 CATALYTIC) (LC17-NM)
8	419	5/46 (10%)	16987.4 / 4.52	CHICK	P02607	MYOSIN LIGHT CHAIN ALKALI, SMOOTH-MUSCLE ISOFORM (GIZZARD) (G2 CATALYTIC) (LC17-GI)
9	391	4/46 (8%)	38545.3 / 8.59	XENLA	P27006	ANNEXIN II TYPE I (LIPOCORTIN II) (CALPACTIN I HEAVY CHAIN) (CHROMOBINDIN 8) (P36) (PROTEIN I) (PLACENTAL ANTICOAGULANT PROTEIN IV) (PAP-IV)
10	286	5/46 (10%)	22156.3 / 5.03	RAT	P16409	MYOSIN LIGHT CHAIN 1, SLOW-TWITCH MUSCLE B/VENTRICULAR ISOFORM
11	262	3/46 (6%)	19590.2 / 9.34	BGMV	P05174	AL2 PROTEIN (19.6 KD PROTEIN)
12	220	5/46 (10%)	21932.2 / 5.03	HUMAN	P08590	MYOSIN LIGHT CHAIN 1, SLOW-TWITCH MUSCLE B/VENTRICULAR ISOFORM (MLC1SB) (ALKALI)
13	211	3/46 (6%)	16990.5 / 6.92	ECOLI	P37052	HYPOTHETICAL 17.0 KDA PROTEIN IN HNR-PURU INTERGENIC REGION
14	202	3/46 (6%)	17947.3 / 5.24	ARATH	P25855	GLYCINE CLEAVAGE SYSTEM H PROTEIN 1, MITOCHONDRIAL PRECURSOR
15	186	3/46 (6%)	16613.9 / 4.63	RAT	P02601	MYOSIN LIGHT CHAIN 3, SKELETAL MUSCLE ISOFORM (A2 CATALYTIC) (ALKALI) (MLC3F)



Detailed Results

1. 9/46 matches (19%). 16930.2 Da, pI = 4.56. Acc. # P16475. HUMAN. MYOSIN LIGHT CHAIN ALKALI, NON-MUSCLE ISOFORM (MLC3NM) (LC17A) (LC17-NM).

m/z submitted	MH$^+$ matched	Delta ppm	start	end	Peptide Sequence (Click for Fragment Ions)	Modifications
995.5787	995.5890	-10.3014	111	119	(R)HVLVTLGEK(M)	
1025.4959	1025.5056	-9.4785	14	21	(K)EAFQLFDR(T)	
1233.5911	1233.5898	1.0857	99	110	(K)EGNGTVMGAEIR(H)	
1354.7187	1354.7331	-10.5955	38	50	(R)ALGQNPTNAEVLK(V)	
1544.6928	1544.6869	3.8248	82	94	(K)DQGTYEDYVEGLR(V)	
1722.8598	1722.8485	6.5620	95	110	(R)VFDKEGNGTVMGAEIR(H)	
1786.8229	1786.8248	-1.0535	80	94	(K)NKDQGTYEDYVEGLR(V)	
1888.0274	1888.0043	12.2526	64	79	(K)VLDFEHFLPMLQTVAK(N)	
2226.1294	2226.1552	-11.6082	99	119	(K)EGNGTVMGAEIRHVLVTLGEK(M)	1Met-ox

37 unmatched masses: 905.6874 973.5183 989.6093 1007.4948 1024.4374 1025.7433 1037.5184 1045.5657 1090.5471 1106.5649 1139.5205 1164.5909 1165.5664 1179.6002 1184.5958 1193.6111 1234.6510 1263.6858 1267.7091 1277.7065 1300.5432 1307.6644 1308.6596 1340.6612 1341.6288 1357.6707 1373.6434 1475.7257 1493.7172 1532.6160 1699.8525 1707.7833 1716.8276 1723.8256 1838.9438 1993.9497 2211.1041

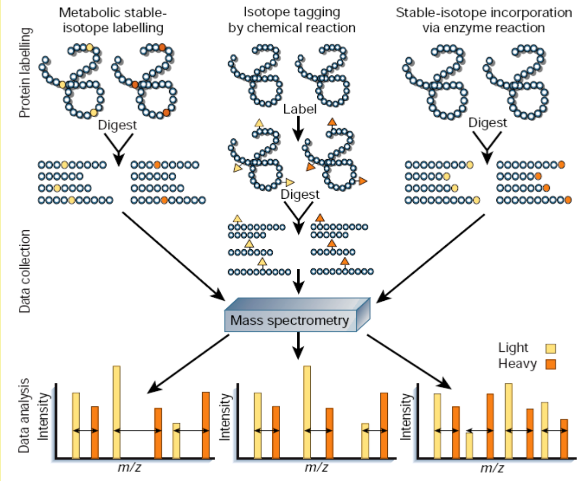
The matched peptides cover **50%** (77/151 AA's) of the protein.
Coverage Map for This Hit (MS-Digest index #): 11572

2. 9/46 matches (19%). 16961.2 Da, pI = 4.46. Acc. # P24572. HUMAN. MYOSIN LIGHT CHAIN ALKALI, SMOOTH-MUSCLE ISOFORM (MLC3SM) (LC17B) (LC17-GI).

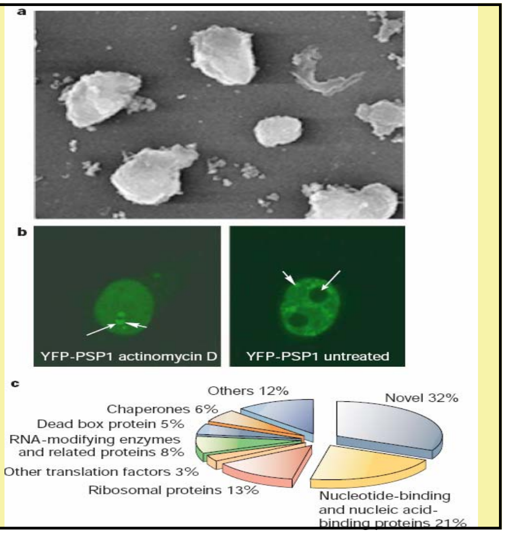
m/z submitted	MH$^+$ matched	Delta ppm	start	end	Peptide Sequence (Click for Fragment Ions)	Modifications
995.5787	995.5890	-10.3014	111	119	(R)HVLVTLGEK(M)	
1025.4959	1025.5056	-9.4785	14	21	(K)EAFQLFDR(T)	
1233.5911	1233.5898	1.0857	99	110	(K)EGNGTVMGAEIR(H)	
1354.7187	1354.7331	-10.5955	38	50	(R)ALGQNPTNAEVLK(V)	
1544.6928	1544.6869	3.8248	82	94	(K)DQGTYEDYVEGLR(V)	
1722.8598	1722.8485	6.5620	95	110	(R)VFDKEGNGTVMGAEIR(H)	
1786.8229	1786.8248	-1.0535	80	94	(K)NKDQGTYEDYVEGLR(V)	
1888.0274	1888.0043	12.2526	64	79	(K)VLDFEHFLPMLQTVAK(N)	
2226.1294	2226.1552	-11.6082	99	119	(K)EGNGTVMGAEIRHVLVTLGEK(M)	1Met-ox

37 unmatched masses: 905.6874 973.5183 989.6093 1007.4948 1024.4374 1025.7433 1037.5184 1045.5657 1090.5471 1106.5649 1139.5205 1164.5909 1165.5664 1179.6002 1184.5958 1193.6111 1234.6510 1263.6858 1267.7091 1277.7065 1300.5432 1307.6644 1308.6596 1340.6612 1341.6288 1357.6707 1373.6434 1475.7257 1493.7172 1532.6160 1699.8525 1707.7833 1716.8276 1723.8256 1838.9438 1993.9497 2211.1041

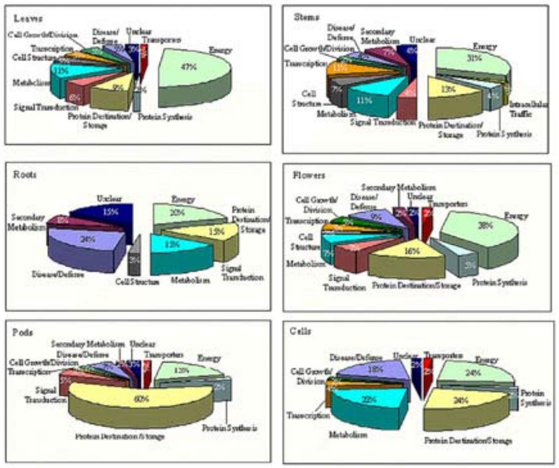
Stable-isotope Protein Labelling for Proteomics



Organellar Proteomics: Combined MS and Imaging Methods



Summary of the functions of various proteins identified in specific tissues of *M. truncatula*.



A Mammalian Organelle Map by Protein Correlation Profiling

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SUMMARY

Protein localization to membrane-enclosed organelles is a central feature of cellular organization. Using protein correlation profiling, we have mapped 1,404 proteins to ten subcellular locations in mouse liver, and these correspond with enzymatic assays, marker protein profiles, and confocal microscopy. These localizations allowed assessment of the specificity in published organellar proteomic inventories and demonstrate multiple locations for 39% of all organellar proteins. Integration of proteomic and genomic data enabled us to identify networks of coexpressed genes, *cis*-regulatory motifs, and putative transcriptional regulators involved in organelle biogenesis. Our analysis ties biochemistry, cell biology, and genomics into a common framework for organelle analysis.

microscopic examination of an organelle, certain proteins or enzyme activities that appear to localize exclusively to that organelle are considered markers, essentially defining that compartment.

Recently, proteomics (de Hoog and Mann, 2004) has been applied to study organelle composition. The genetic tractability of *Saccharomyces cerevisiae* has allowed a large fraction of yeast ORFs to be tagged for localization studies (Ross-Macdonald et al., 1999; Kumar et al., 2002; Huh et al., 2003), but such an approach is more challenging in mammalian systems due, in part, to artifacts from overexpression (Simpson et al., 2000). Mass spectrometry-based proteomics (Aebersold and Mann, 2003) is often employed to characterize the protein composition of organelle-enriched fractions. Indeed, protein catalogs are now available for virtually all cytoplasmic organelles as well as most of the major nuclear ones (reviewed in Yates et al., 2005). However, due to the high sensitivity of mass spectrometers and the difficulties inherent in purifying organelles to homogeneity, it has been challenging

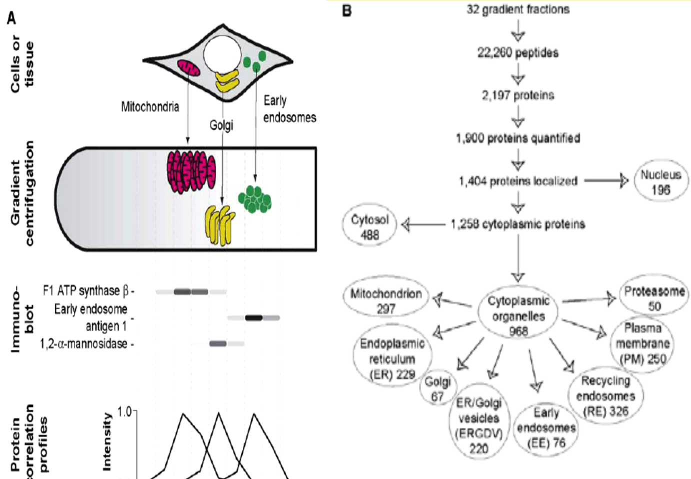
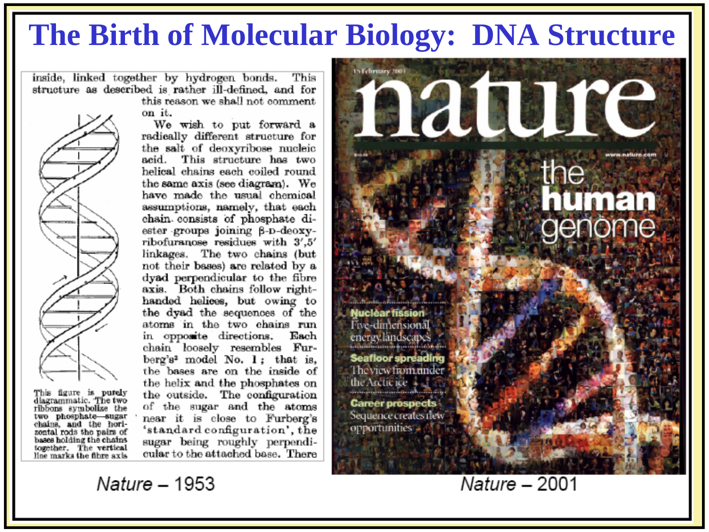


Figure 1. Organelle Profiling with Gradient Centrifugation



The Birth of Molecular Biology: DNA Structure

inside, linked together by hydrogen bonds. This structure as described is rather ill-defined, and for this reason we shall not comment on it.

We wish to put forward a radically different structure for the salt of deoxyribose nucleic acid. This structure has two helical chains each coiled round the same axis (see diagram). We have made the usual chemical assumptions, namely, that each chain consists of phosphate diester groups joining β-D-deoxyribofuranose residues with 3′,5′ linkages. The two chains (but not their bases) are related by a dyad perpendicular to the fibre axis. Both chains follow right-handed helices, but owing to the dyad the sequences of the atoms in the two chains run in opposite directions. Each chain loosely resembles Furberg's[2] model No. 1; that is, the bases are on the inside of the helix and the phosphates on the outside. The configuration of the sugar and the atoms near it is close to Furberg's 'standard configuration', the sugar being roughly perpendicular to the attached base. There

This figure is purely diagrammatic. The two ribbons symbolize the two phosphate—sugar chains, and the horizontal rods the pairs of bases holding the chains together. The vertical line marks the fibre axis

Nature – 1953

15 February 2001
nature
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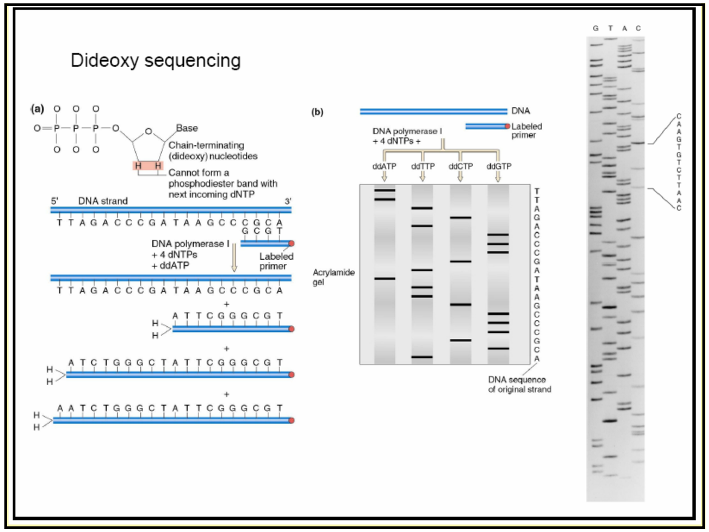
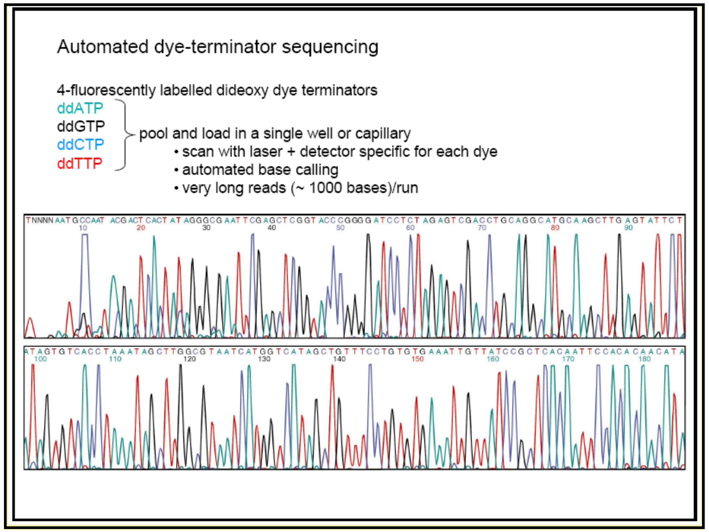
the human genome
Nuclear fission
Five-dimensional energy landscapes
Seafloor spreading
The view from under the Arctic ice
Career prospects
Sequence creates new opportunities

Nature – 2001



Dideoxy sequencing

(a)
O=P—P—P—O
Base
Chain-terminating (dideoxy) nucleotides
H H
Cannot form a phosphodiester band with next incoming dNTP
5' DNA strand 3'
T T A G A C C C G A T A A G C C C G C A
G C G T
DNA polymerase I
+ 4 dNTPs
+ ddATP
Labeled primer
T T A G A C C C G A T A A G C C C G C A
+
A T T C G G G C G T
+
A T C T G G G C T A T T C G G G C G T
+
A A T C T G G G C T A T T C G G G C G T
(b)
DNA
Labeled primer
DNA polymerase I
+ 4 dNTPs +
ddATP ddTTP ddCTP ddGTP
Acrylamide gel
T T A G A C C C G A T A A G C C C G C A
DNA sequence of original strand
G T A C
C A A G T G T C T T A A C

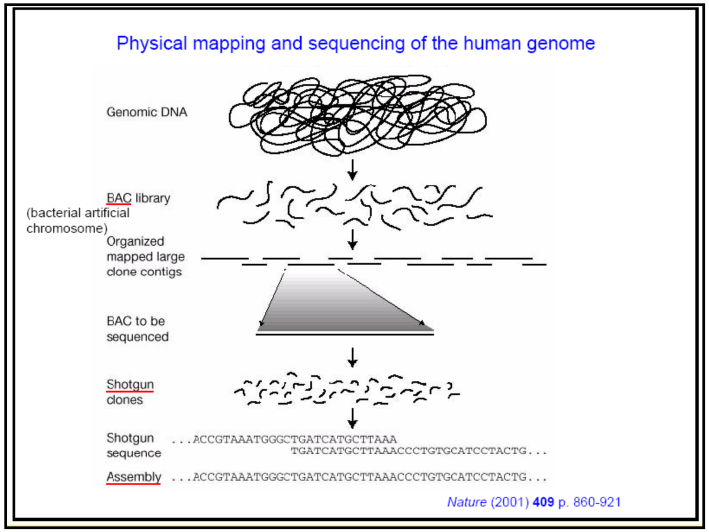


Automated dye-terminator sequencing

4-fluorescently labelled dideoxy dye terminators

ddATP
ddGTP
ddCTP
ddTTP

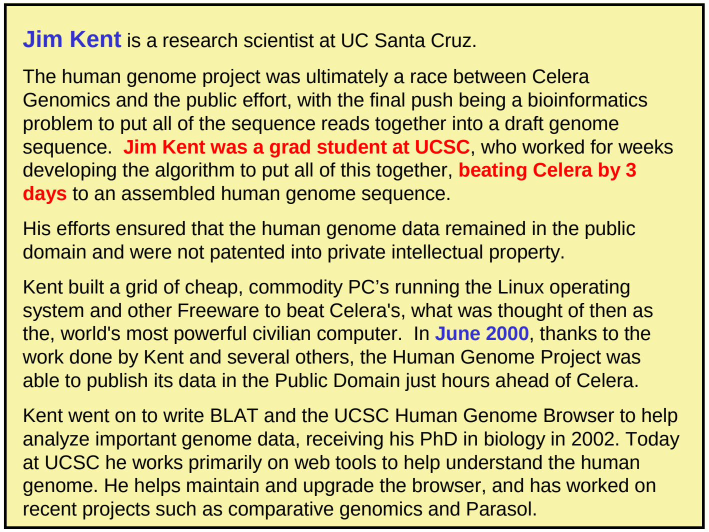
pool and load in a single well or capillary
- scan with laser + detector specific for each dye
- automated base calling
- very long reads (~ 1000 bases)/run



Physical mapping and sequencing of the human genome

Genomic DNA
BAC library
(bacterial artificial chromosome)
Organized mapped large clone contigs
BAC to be sequenced
Shotgun clones
Shotgun sequence
...ACCGTAAATGGGCTGATCATGCTTAAA
TGATCATGCTTAAACCCTGTGCATCCTACTG...
Assembly
...ACCGTAAATGGGCTGATCATGCTTAAACCCTGTGCATCCTACTG...

Nature (2001) **409** p. 860-921



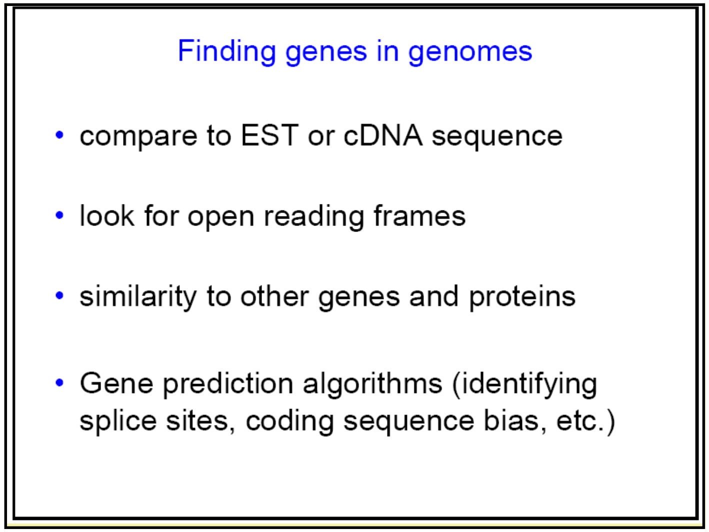
Jim Kent is a research scientist at UC Santa Cruz.

The human genome project was ultimately a race between Celera Genomics and the public effort, with the final push being a bioinformatics problem to put all of the sequence reads together into a draft genome sequence. **Jim Kent was a grad student at UCSC**, who worked for weeks developing the algorithm to put all of this together, **beating Celera by 3 days** to an assembled human genome sequence.

His efforts ensured that the human genome data remained in the public domain and were not patented into private intellectual property.

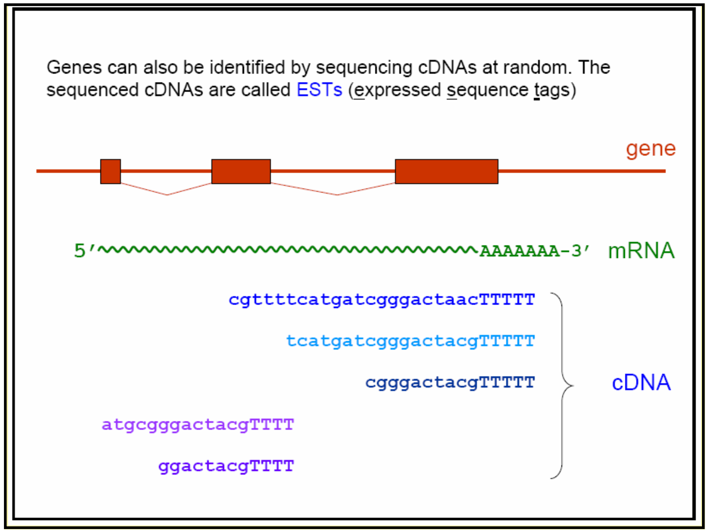
Kent built a grid of cheap, commodity PC's running the Linux operating system and other Freeware to beat Celera's, what was thought of then as the, world's most powerful civilian computer. In **June 2000**, thanks to the work done by Kent and several others, the Human Genome Project was able to publish its data in the Public Domain just hours ahead of Celera.

Kent went on to write BLAT and the UCSC Human Genome Browser to help analyze important genome data, receiving his PhD in biology in 2002. Today at UCSC he works primarily on web tools to help understand the human genome. He helps maintain and upgrade the browser, and has worked on recent projects such as comparative genomics and Parasol.

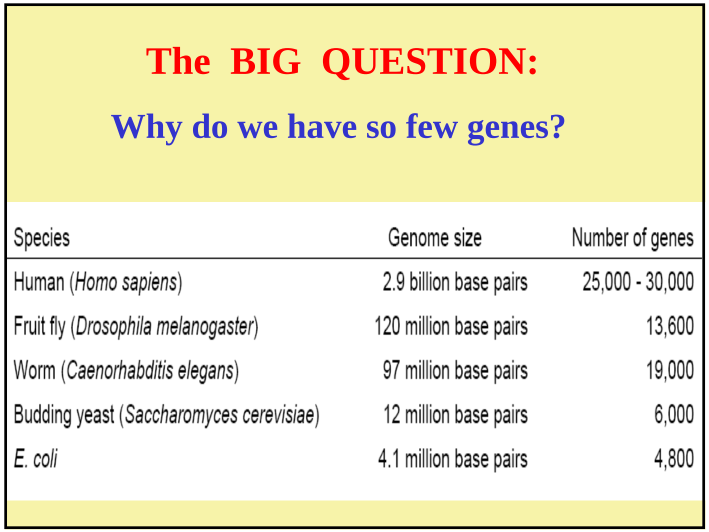


Finding genes in genomes

- compare to EST or cDNA sequence
- look for open reading frames
- similarity to other genes and proteins
- Gene prediction algorithms (identifying splice sites, coding sequence bias, etc.)



Genes can also be identified by sequencing cDNAs at random. The sequenced cDNAs are called ESTs (expressed sequence tags)



The BIG QUESTION:

Why do we have so few genes?

Species	Genome size	Number of genes
Human (*Homo sapiens*)	2.9 billion base pairs	25,000 - 30,000
Fruit fly (*Drosophila melanogaster*)	120 million base pairs	13,600
Worm (*Caenorhabditis elegans*)	97 million base pairs	19,000
Budding yeast (*Saccharomyces cerevisiae*)	12 million base pairs	6,000
E. coli	4.1 million base pairs	4,800

Genomics vs. Proteomics

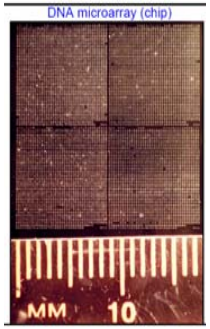
With the completion of a rough draft of the human genome, many researchers are looking at how genes and proteins interact to form other proteins. A surprising finding of the Human Genome Project is that there are far fewer protein-coding genes in the human genome than proteins in the human proteome (20,000 to 25,000 genes vs. about 1,000,000 proteins). The human body may contain more than 2 million proteins, each having different functions. The protein diversity is thought to be due to alternative splicing and post-translational modification of proteins. The discrepancy implies that protein diversity cannot be fully characterized by gene expression analysis, thus proteomics is useful for characterizing cells and tissues.

Functional genomics and proteomics

- Identify genes and proteins encoded in the genome (Gene finding)
- Measure gene expression on a genome-wide scale (microarrays)
- Identify protein function
  30-50% of the genes in a genome are of unknown function
- Identify protein interactions, biochemical pathways, gene interaction networks inside cells

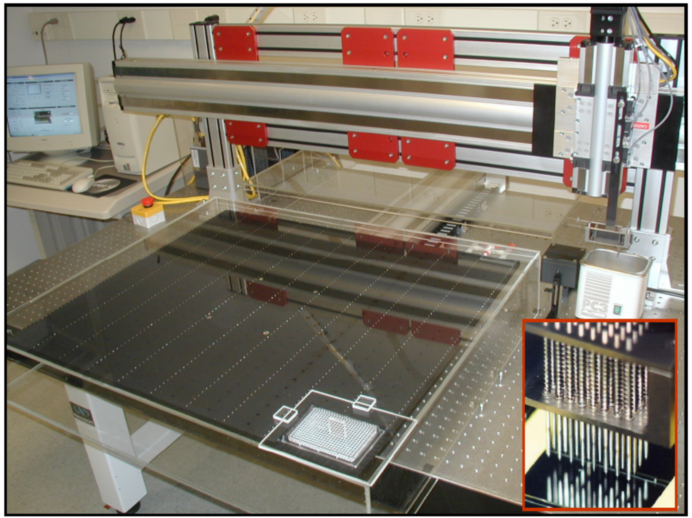
Methods of making microarrays

- Robotic spotting
  - using a printing tip
  - using inkjets
- Synthesis of oligonucleotides
  - photolithography (Affymetrix)
  - using inkjets
  - Digital Light Processor (DLP) or Digital Micromirror Device (DMD)

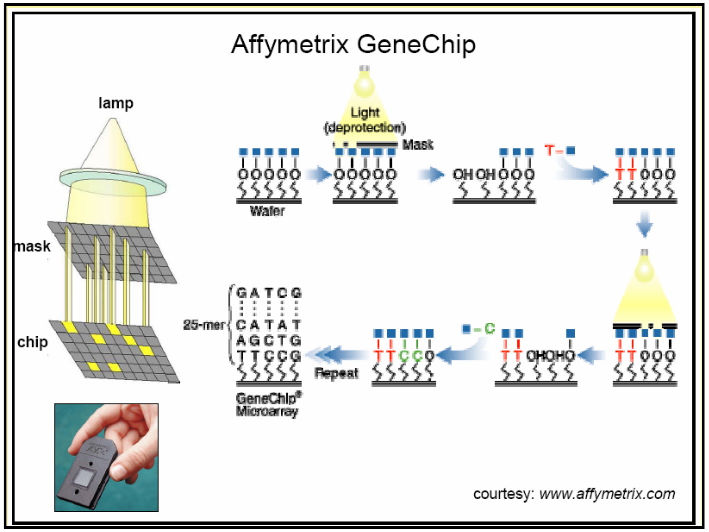


Microarrays can be used to study gene expression, DNA-protein interactions, mutations, protein-protein interactions, etc., all on a genome-wide scale

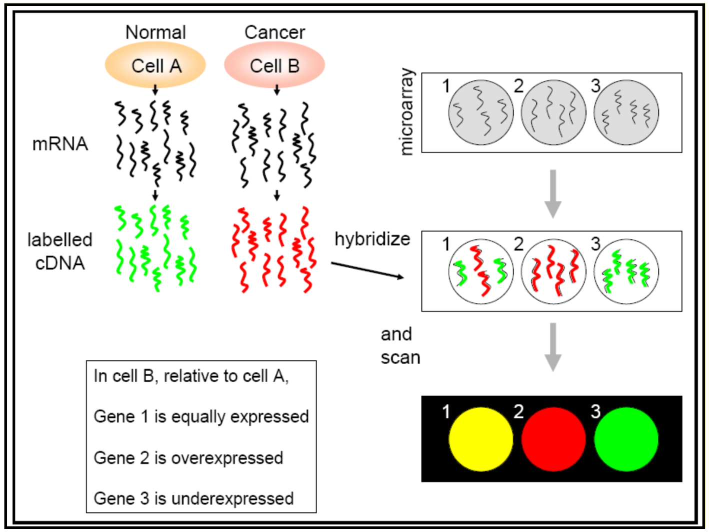
Note: Thanks to Prof. Vishy Iyer for many of these slides on microarrrays.



Affymetrix GeneChip



courtesy: *www.affymetrix.com*

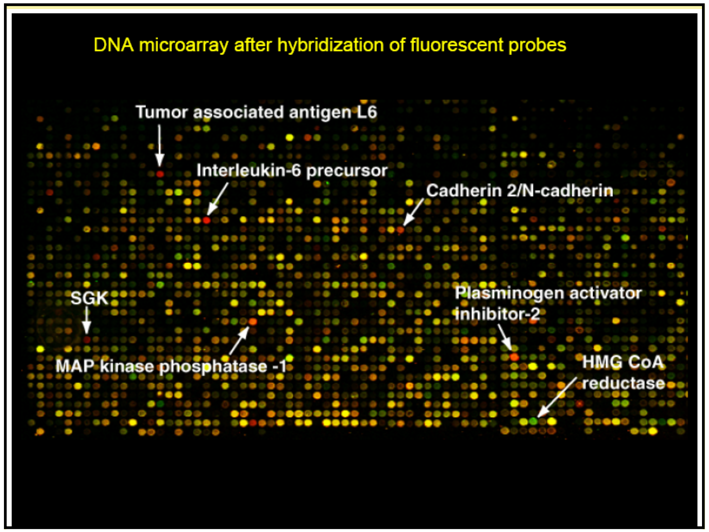


In cell B, relative to cell A,

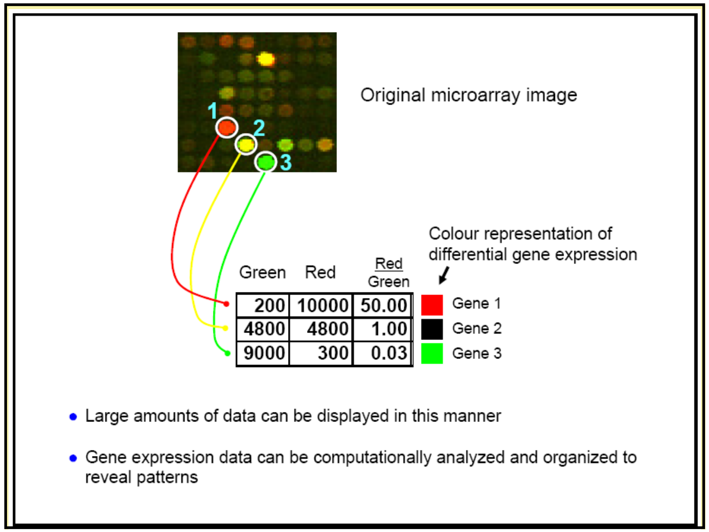
Gene 1 is equally expressed

Gene 2 is overexpressed

Gene 3 is underexpressed



DNA microarray after hybridization of fluorescent probes

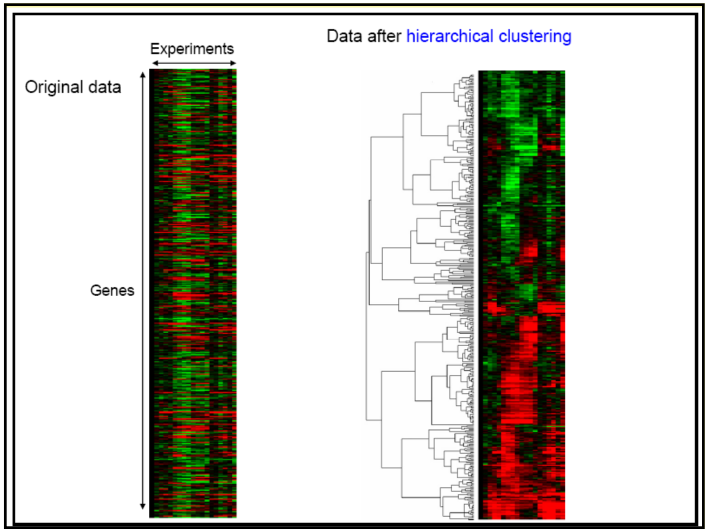


Original microarray image

Colour representation of differential gene expression

Green	Red	Red/Green	
200	10000	50.00	🟥 Gene 1
4800	4800	1.00	■ Gene 2
9000	300	0.03	🟩 Gene 3

- Large amounts of data can be displayed in this manner
- Gene expression data can be computationally analyzed and organized to reveal patterns

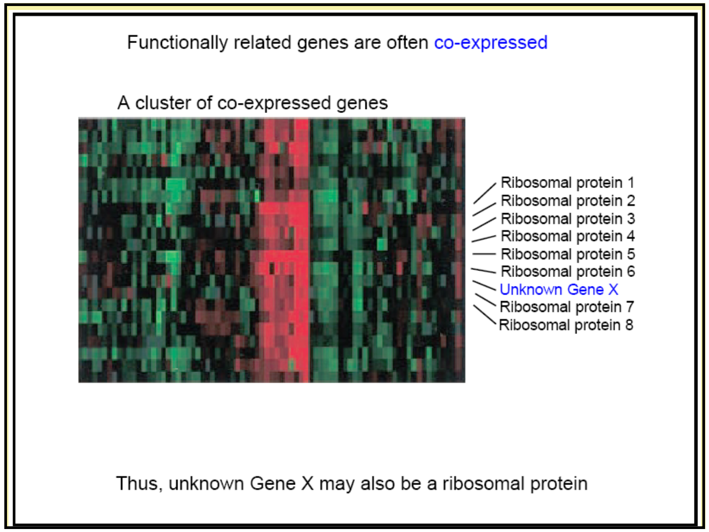


Data after hierarchical clustering

Original data

Experiments

Genes

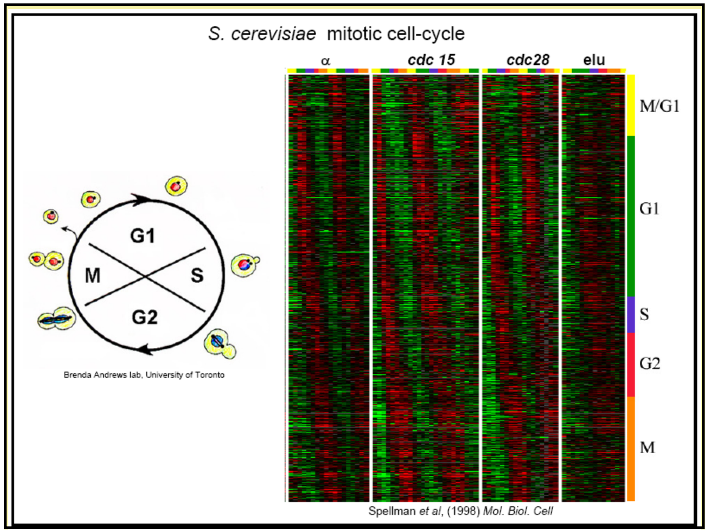


Functionally related genes are often co-expressed

A cluster of co-expressed genes

Ribosomal protein 1
Ribosomal protein 2
Ribosomal protein 3
Ribosomal protein 4
Ribosomal protein 5
Ribosomal protein 6
Unknown Gene X
Ribosomal protein 7
Ribosomal protein 8

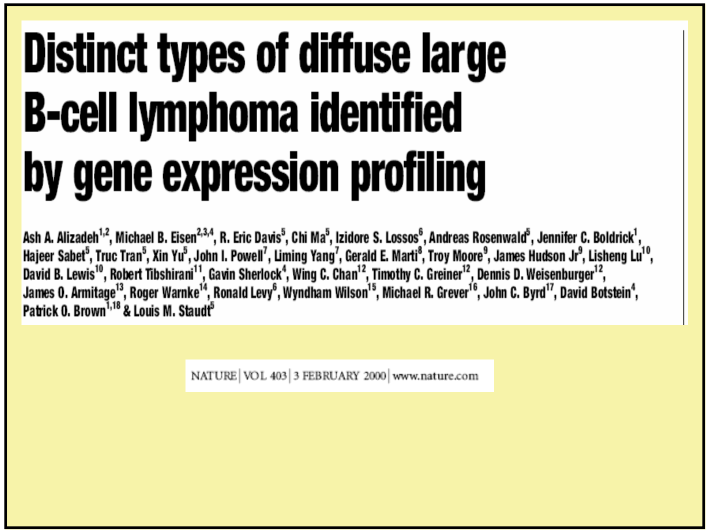
Thus, unknown Gene X may also be a ribosomal protein



S. cerevisiae mitotic cell-cycle

Brenda Andrews lab, University of Toronto

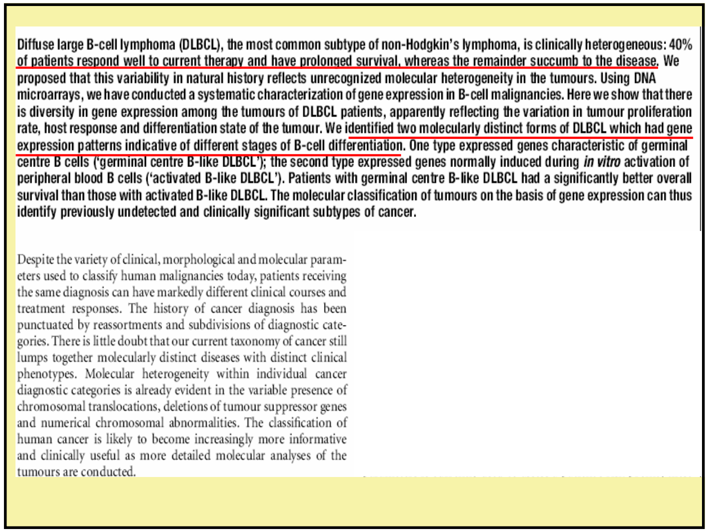
Spellman *et al*, (1998) *Mol. Biol. Cell*



Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling

Ash A. Alizadeh[1,2], Michael B. Eisen[2,3,4], R. Eric Davis[5], Chi Ma[5], Izidore S. Lossos[6], Andreas Rosenwald[5], Jennifer C. Boldrick[1], Hajeer Sabet[5], Truc Tran[5], Xin Yu[5], John I. Powell[7], Liming Yang[7], Gerald E. Marti[8], Troy Moore[9], James Hudson Jr[9], Lisheng Lu[10], David B. Lewis[10], Robert Tibshirani[11], Gavin Sherlock[4], Wing C. Chan[12], Timothy C. Greiner[12], Dennis D. Weisenburger[12], James O. Armitage[13], Roger Warnke[14], Ronald Levy[6], Wyndham Wilson[15], Michael R. Grever[16], John C. Byrd[17], David Botstein[4], Patrick O. Brown[1,18] & Louis M. Staudt[5]

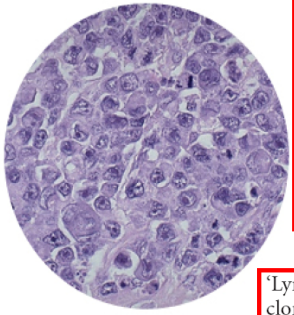
NATURE | VOL 403 | 3 FEBRUARY 2000 | www.nature.com



Diffuse large B-cell lymphoma (DLBCL), the most common subtype of non-Hodgkin's lymphoma, is clinically heterogeneous: 40% of patients respond well to current therapy and have prolonged survival, whereas the remainder succumb to the disease. We proposed that this variability in natural history reflects unrecognized molecular heterogeneity in the tumours. Using DNA microarrays, we have conducted a systematic characterization of gene expression in B-cell malignancies. Here we show that there is diversity in gene expression among the tumours of DLBCL patients, apparently reflecting the variation in tumour proliferation rate, host response and differentiation state of the tumour. We identified two molecularly distinct forms of DLBCL which had gene expression patterns indicative of different stages of B-cell differentiation. One type expressed genes characteristic of germinal centre B cells ('germinal centre B-like DLBCL'); the second type expressed genes normally induced during *in vitro* activation of peripheral blood B cells ('activated B-like DLBCL'). Patients with germinal centre B-like DLBCL had a significantly better overall survival than those with activated B-like DLBCL. The molecular classification of tumours on the basis of gene expression can thus identify previously undetected and clinically significant subtypes of cancer.

Despite the variety of clinical, morphological and molecular parameters used to classify human malignancies today, patients receiving the same diagnosis can have markedly different clinical courses and treatment responses. The history of cancer diagnosis has been punctuated by reassortments and subdivisions of diagnostic categories. There is little doubt that our current taxonomy of cancer still lumps together molecularly distinct diseases with distinct clinical phenotypes. Molecular heterogeneity within individual cancer diagnostic categories is already evident in the variable presence of chromosomal translocations, deletions of tumour suppressor genes and numerical chromosomal abnormalities. The classification of human cancer is likely to become increasingly more informative and clinically useful as more detailed molecular analyses of the tumours are conducted.

The challenge of cancer diagnosis



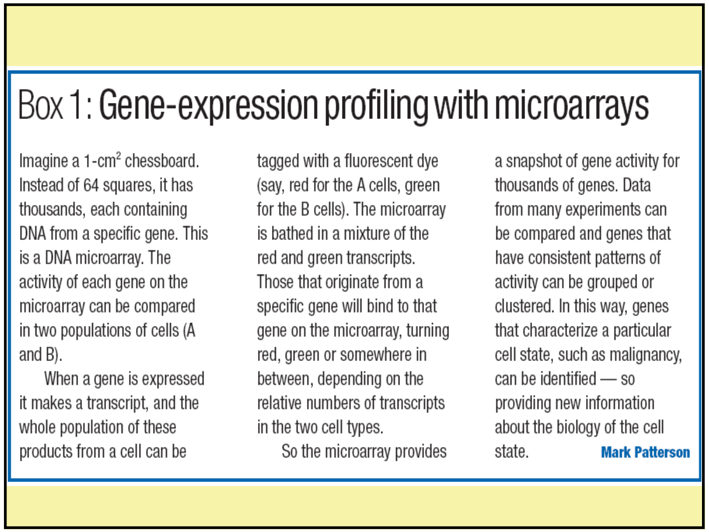
Diffuse large B-cell lymphoma is the most common subtype of non-Hodgkin's lymphoma. With current treatments, long-term survival can be achieved in only 40% of patients. There are no reliable indicators — morphological, clinical, immunohistochemical or genetic — that can be used to recognize subclasses of **DLBCL** and point to a differential therapeutic approach to patients.

'Lymphochip', a microarray carrying 18,000 clones of complementary DNA designed to monitor genes involved in normal and abnormal lymphocyte development.

What type of cancer?

What is the underlying molecular basis?

What is the optimal treatment?



Box 1: Gene-expression profiling with microarrays

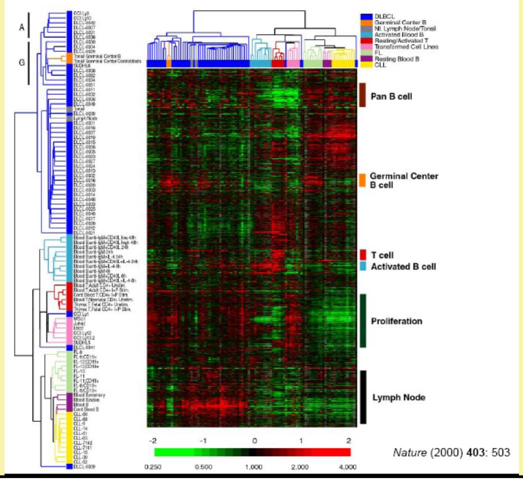
Imagine a 1-cm^2 chessboard. Instead of 64 squares, it has thousands, each containing DNA from a specific gene. This is a DNA microarray. The activity of each gene on the microarray can be compared in two populations of cells (A and B).

When a gene is expressed it makes a transcript, and the whole population of these products from a cell can be tagged with a fluorescent dye (say, red for the A cells, green for the B cells). The microarray is bathed in a mixture of the red and green transcripts. Those that originate from a specific gene will bind to that gene on the microarray, turning red, green or somewhere in between, depending on the relative numbers of transcripts in the two cell types.

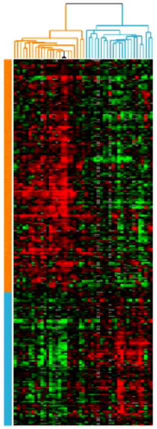
So the microarray provides a snapshot of gene activity for thousands of genes. Data from many experiments can be compared and genes that have consistent patterns of activity can be grouped or clustered. In this way, genes that characterize a particular cell state, such as malignancy, can be identified — so providing new information about the biology of the cell state.

Mark Patterson

Hierarchical clustering of gene expression data (as ratios).

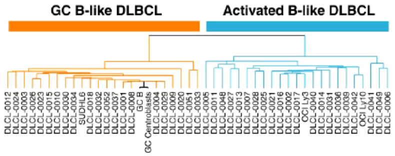


Nature (2000) **403**: 503

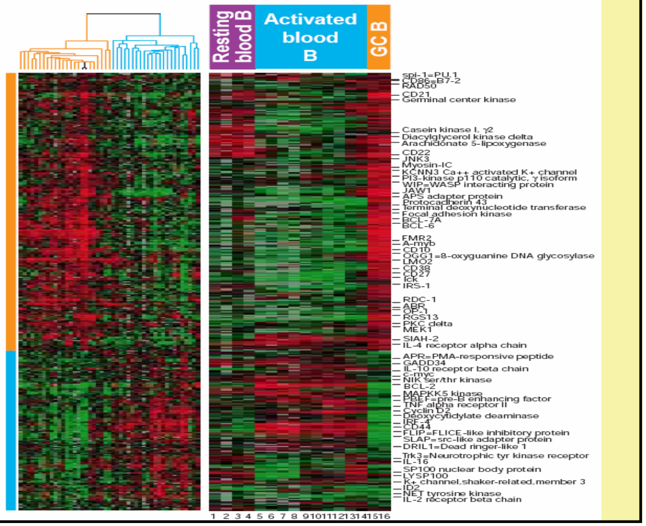


Clustering of tumour samples from cancer patients can be used for molecular classification of cancers. This may be useful for diagnosis and treatment

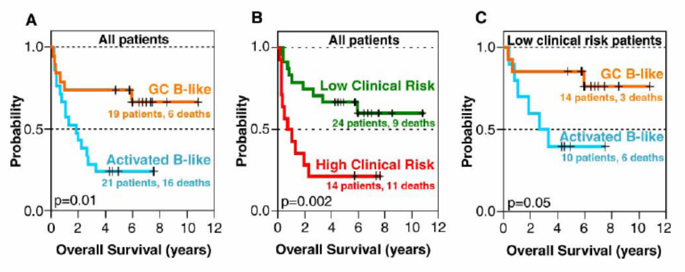
Subtypes of Diffuse Large B-Cell Lymphoma (DLBCL)



Nature (2000) **403**: 503

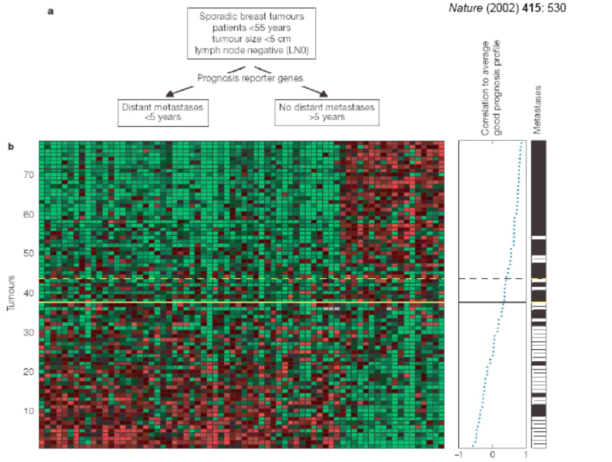


Using "clustering analysis," Alizadeh *et al.* could separate DLBCL into two categories, which had marked differences in overall survival of the patients concerned. The gene expression signatures of these subgroups corresponded to distinct stages in the differentiation of B cells, the type of lymphocyte that makes antibodies.

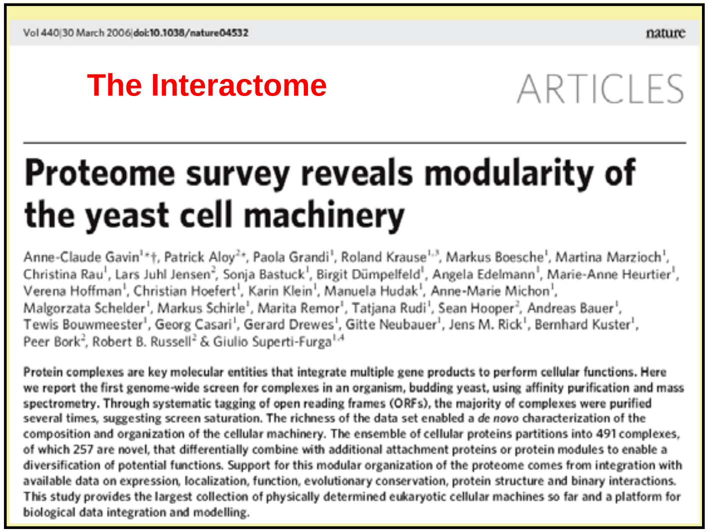


Identifying a gene expression signature for breast cancer metastasis

Nature (2002) **415**: 530



Nature (2002) **415**: 530



Vol 440|30 March 2006|doi:10.1038/nature04532 nature

The Interactome

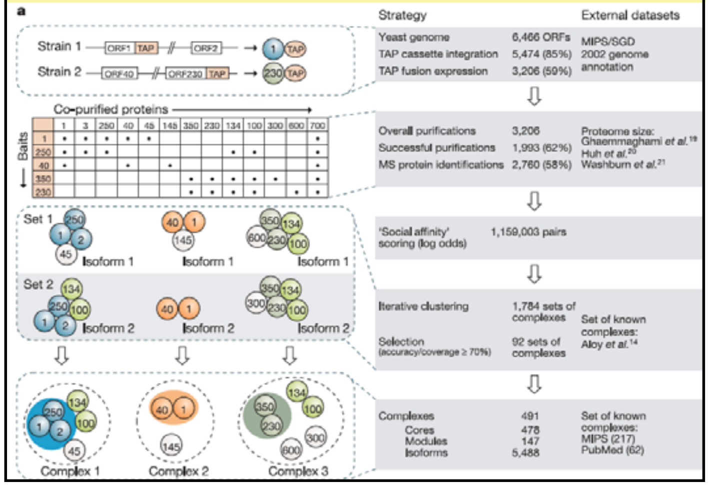
ARTICLES

Proteome survey reveals modularity of the yeast cell machinery

Anne-Claude Gavin[1]*†, Patrick Aloy[2]*, Paola Grandi[1], Roland Krause[1,3], Markus Boesche[1], Martina Marzioch[1], Christina Rau[1], Lars Juhl Jensen[2], Sonja Bastuck[1], Birgit Dümpelfeld[1], Angela Edelmann[1], Marie-Anne Heurtier[1], Verena Hoffman[1], Christian Hoefert[1], Karin Klein[1], Manuela Hudak[1], Anne-Marie Michon[1], Malgorzata Schelder[1], Markus Schirle[1], Marita Remor[1], Tatjana Rudi[1], Sean Hooper[2], Andreas Bauer[1], Tewis Bouwmeester[1], Georg Casari[1], Gerard Drewes[1], Gitte Neubauer[1], Jens M. Rick[1], Bernhard Kuster[1], Peer Bork[2], Robert B. Russell[2] & Giulio Superti-Furga[1,4]

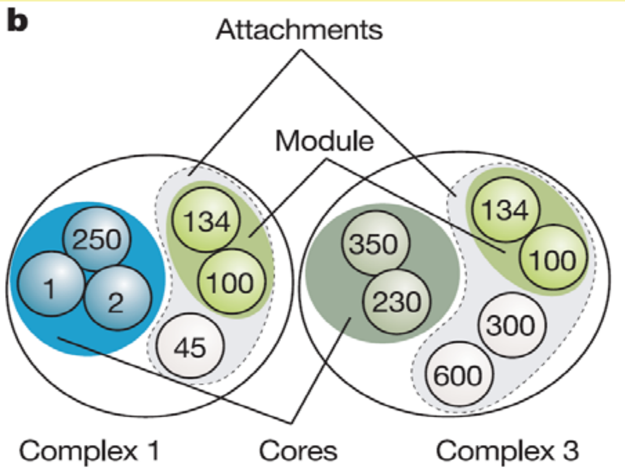
Protein complexes are key molecular entities that integrate multiple gene products to perform cellular functions. Here we report the first genome-wide screen for complexes in an organism, budding yeast, using affinity purification and mass spectrometry. Through systematic tagging of open reading frames (ORFs), the majority of complexes were purified several times, suggesting screen saturation. The richness of the data set enabled a *de novo* characterization of the composition and organization of the cellular machinery. The ensemble of cellular proteins partitions into 491 complexes, of which 257 are novel, that differentially combine with additional attachment proteins or protein modules to enable a diversification of potential functions. Support for this modular organization of the proteome comes from integration with available data on expression, localization, function, evolutionary conservation, protein structure and binary interactions. This study provides the largest collection of physically determined eukaryotic cellular machines so far and a platform for biological data integration and modelling.

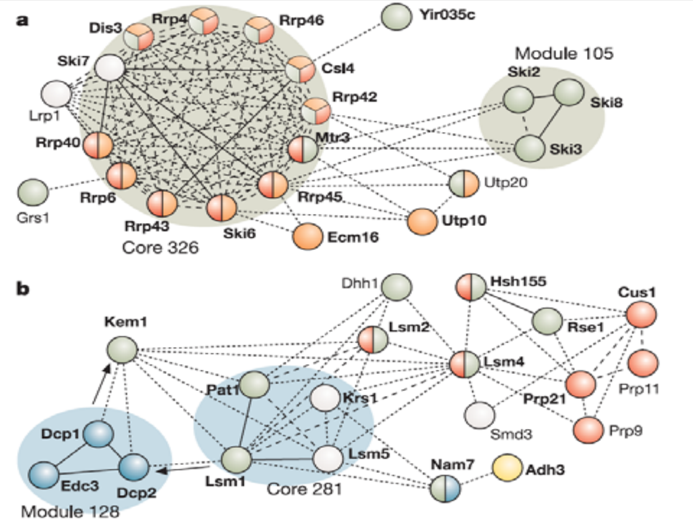
Architecture and Modularity of Complexes



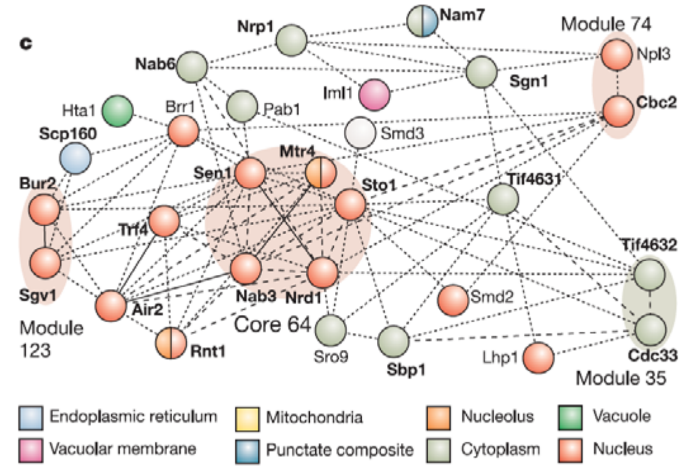
	1	3	250	40	45	145	350	230	134	100	300	600	700
1	•	•	•	•	•								•
250	•	•	•						•	•			•
40	•			•		•							•
350							•	•	•	•	•		•
230							•	•	•	•		•	•

Strategy		External datasets
Yeast genome	6,466 ORFs	MIPS/SGD 2002 genome annotation
TAP cassette integration	5,474 (85%)	
TAP fusion expression	3,206 (59%)	
Overall purifications	3,206	Proteome size: Ghaemmaghami *et al.*[19] Huh *et al.*[20] Washburn *et al.*[21]
Successful purifications	1,993 (62%)	
MS protein identifications	2,760 (58%)	
'Social affinity' scoring (log odds)	1,159,003 pairs	
Iterative clustering	1,784 sets of complexes	Set of known complexes: Aloy *et al.*[14]
Selection (accuracy/coverage ≥ 70%)	92 sets of complexes	
Complexes	491	Set of known complexes: MIPS (217) PubMed (62)
Cores	478	
Modules	147	
Isoforms	5,488	

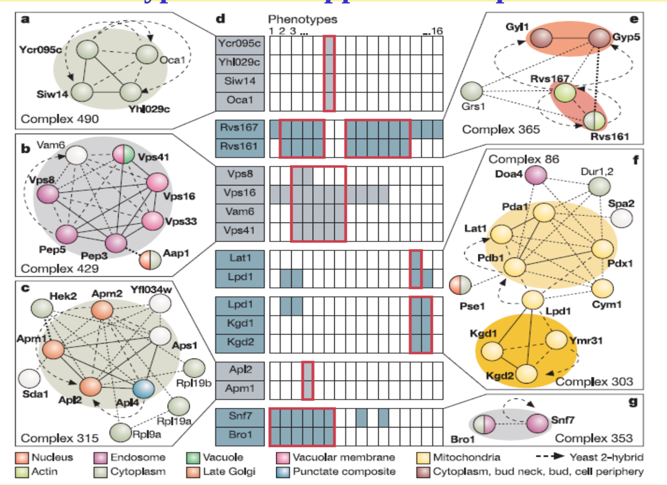




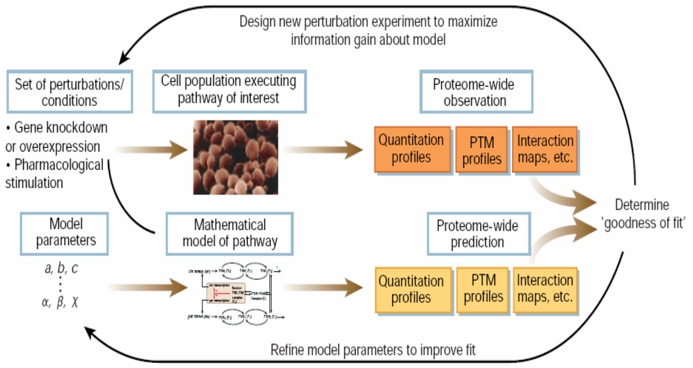
Architecture and Modularity of Complexes



Phenotypic Data Mapped to Complexes



Systems Biology Approach



Design new perturbation experiment to maximize information gain about model

Set of perturbations/ conditions

Cell population executing pathway of interest

Proteome-wide observation

- Gene knockdown or overexpression
- Pharmacological stimulation

Quantitation profiles | PTM profiles | Interaction maps, etc.

Determine 'goodness of fit'

Model parameters

a, *b*, *c*
⋮
α, *β*, *χ*

Mathematical model of pathway

Proteome-wide prediction

Quantitation profiles | PTM profiles | Interaction maps, etc.

Refine model parameters to improve fit