Genome-wide Gene Expression Profiling in Fission Yeast http://www.sanger.ac.uk/PostGenomics/S pombe Jürg Bähler **The Wellcome Trust Sanger Institute / Cancer Research UK**

Post-genomic vs traditional experiments:

Genes or gene products:	1 2 3	n
Gene cloning	1 1 1	
Gene expression •	Horizontal approach	\rightarrow
Gene deletion		
Protein localization	al app	
Protein interactions	roach	
Enzymatic activities	↓ ▼	

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Schizosaccharomyces pombe (Fission Yeast)



- unicellular eukaryote (fungus)
- genome recently sequenced: ~14 Mb, <5,000 genes
- easy to handle / genetics
- evolutionary distant to S. cerevisiae
- no beauty but what a beast!

Primer Design

- Annealing temperature: 58-62°C; GC content: 40–60%
- Product length: 200–500 bp; <2500 bp from gene end
- **Products are 100% exon sequence**
- Products have Blast score <400 with other fission yeast sequences
- All ORFs (nuclear and mitochondrial), 'pseudogenes', introns, bacterial control genes



Unspecific hybridisation to similar probes:



5'-aminolink surface chemistry

- covalent attachment of DNA via 5'-aminolink modification
- single-stranded probes can be made after attachment



probes can distinguish transcriptional direction
 entire length of probe accessible for hybridization
 high sensitivity



The fission yeast genome on a microarray

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6050 spots printed in duplicate: 12,100 spots

Data Processing Pipeline

- Image Analysis Software: determine signal ratios (e.g., GenePix)
- InHouse program for initial data processing: filter weak and irreproducible signals, local normalization, quality control

- Data mining using various software (GeneSpring, Cluster, SAM, ArrayMiner,...)
- Oracle Database MIDAS: Plate tracking and storage of raw data

Local Normalization:

Running window 1000 spots





Accuracy of signal ratios determined by spiking of <i>S. cerevisiae</i> RNA			
Spiked ratios	Measured median ratios (range)	Range of signals	
1:2	1.9 (1.8 - 2.1)	490/270 – 2,080/32,060	
1:5	5.5 (4.3 - 7.2)	420/90 – 58,320/10,990	
1:10	9.7 (5.8 - 11.1)	380/40 – 35,890/5620	
1:20	19.6 (12.2 - 23.6)	340/20 – 56,900/3110	

Reproducibility of signal ratios and intensities:



Reproducibility of array data:

Measurement	Mean SD (Range)	CV (Range)
Within array replicates	0.04 (0.03-0.06)	4.4% (3.1-6.2%)
Technical repeats	0.04 (0.02-0.06)	4.5% (2.5-6.3%)
Biological repeats	0.07 (0.05-0.10)	6.4% (4.9-8.1%)

Three main projects:



Expression Profiling During Sexual Differentiation: Juan Mata synchronized meiotic cell cultures transcription factor mutants genome-wide transcriptional program Stage-specific gene expression regulatory sequence motifs and circuits



Vegetative cell cycle

Meiotic timecourses: major clusters of gene expression





Functions of early genes:



Known meiotic transcription factors are themselves regulated:



Other transcription factors up-regulated during meiosis:



Characterization of *atf* **function during meiosis:**



Transcriptional Regulation During Meiosis



Global regulation of genes up-regulated in N-starvation:



Stress Response Mechanisms:

Dongrong Chen Mark Toone, Nic Jones (Paterson, Manchester)

various stresses: heat, osmotic, toxic metals, ...
oxidative stress: time, dose, different oxidants

> general vs specific stress responses
 > acute vs adaptive stress response
 > long-term adaptation to stress
 > regulatory sequence motifs and circuits

Eukaryotic stress-activated protein kinase pathways



Importance of stress and stress response pathways

<u>in human pathology:</u>

- Ethanol induced liver damage
- Ischemic hepatitis
- Ischemia/reperfusion injury catalyzed by Xanthine
 - Oxidase- transplantation biology
 - cerebrovascular injury
- Parkinson's disease
- Amyotrophic lateral sclerosis
- Atherosclerosis
- Ageing (senescence)
- Cancer

<u>Homeostasis:</u>

- Kidney cells exposed to dramatic fluctuations in osmolarity
- Metabolism of toxic compounds in the liver
- Apoptosis
- Immune system
- Resistance to DNA damaging agents

<u>CESR</u>: Core Environmental Stress Response



The transcriptional response to stress consists of general and stress-specific gene induction:



Regulation of stress response genes:



Regulation of stress response genes:



CESR

Non-CESR

Different regulation of stress response genes in two yeasts:

Budding Yeast

Fission Yeast



core stress response genes







Growth media influence stress gene expression:



Increasing cell density and stress gene expression:



Increasing cell density and stress gene expression:



Expression Profiling During Cell Cycle: Gabriella Rustici synchronized cell cultures cell cycle and transcription factor mutants > periodic (stage-specific) gene expression gene function ('guilt by association') regulatory sequence motifs and circuits

All genes: 2 experiments



~200 periodic genes: 7 different experiments



Average expression profiles of four main clusters (7 experiments/158 samples):



4 major waves of transcription:



Average gene expression profiles:



Expression ratios

Minutes after synchronization

Principal component analysis:



Major Conclusions:

• 4 distinct waves of transcription, with gap during much of G2 phase where no genes seem to be regulated

~200 periodic genes (4% of genome):
 26 of those genes have been described as cell-cycle regulated, while majority of remaining genes have unknown function

- Study regulation of periodic cell cycle transcription using various mutants, overexpressors, and Chip-chip
- Compare and contrast with periodically expressed genes in other organisms

General and simple recommendations:

Repeat biological experiments to get statistically sound data

Plan and design experiments carefully / controlled and standardized conditions
 Compare data from different experiments The more data the better!

Explore data with various tools



Thank you!

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