Global Gene Expression Programs 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	↓ ▼	

...

The global view...



Global map as an approximation of reality:



Gene expression data

Evolution has carried out the <u>perfect functional</u> <u>genomic experiment</u>:

a multi-million year, genome-wide conditional knock-out mutagenesis, accompanied by exhaustive phenotypic screens. The results are recorded in gene expression programs activated under various conditions.

Microarrays: Principle of Differential Hybridization

Reference Experiment

RNA Isolation



Scanned Image

Fluorescence ratios





The fission yeast genome on a microarray



6500 spots printed in duplicate: 13,000 spots

Three main projects:



Cell cycle control of gene expression

- Universal level of regulation
- Budding yeast: 400-800 periodically expressed genes
- Fission yeast: ~35 periodic genes reported

- Genome-wide overview of cell cycle-regulated gene expression in fission yeast
- Conservation of periodic gene expression programs?

Periodic Gene Expression during Cell Cycle:



DNA microarrays to study stage-specific gene expression during fission yeast cell cycle

Synchronization of cells in different ways:

centrifugal elutriation conditional cell cycle mutants

All genes: 2 experiments



Identification of periodic genes: autocorrelation script



Combination of wavelet analysis and Fourier Significance: Randomizations Confirmation by visual inspection

Phaseogram: 407 periodically expressed genes



4 major waves of transcription:



Average gene expression profiles:



Principal component analysis:



4 major waves of gene expression:



Major cell cycle transcription factors

Budding yeast:



Regulation of periodically transcribed genes



Transcriptional regulation of clusters 1 and 2



Transcriptional regulation by Sep1p and Ace2p:



timepoints of synchronized cultures

Mutant phenotypes



wild type

 $sep1\Delta$

 $ace2\Delta$

 $ace2\Delta sep1 \Delta$

P. Lindner, 1893



Identification of regulatory promoter motifs:



motif logos: Forkhead TGTTTACA. Novel 1 ᠳᡗᡀᠴ᠍ Novel 2 TgCATT ç MCB 1 CGCGTI MCB 2 CCCG+CCCG_{TC} Ace2 CAGULAT Histone Novel 3 CICCCT

Regulatory gene expression networks:

S



Core cell cycle-regulated genes:



Core cell cycle-regulated genes:

Mitosis and cell division:

CDC5, IPL1, KIN3	plo1, ark1, fin1	Polo, Aurora, and NimA kinases
CDC20	slp1	Activator of APC
SPO12	wis3	Putative cell-cycle regulator
KAR3, KIP1	klp5, klp6, klp8	Kinesin microtubule motor
MOB1, DBF2	mob1, sid2	Proteins involved in MEN/SIN
MYO1	myo3	Myosin II heavy chain
BUD4	mid2	Protein involved in cytokinesis
ACE2	ace2	Transcription factor
HOF1	imp2	Protein involved in cell division
DSE4	eng1	Glucanase for cell separation
CHS2	chs2	Protein involved in septum formation
TOS7	mac1	Putative role in cell separation

Core cell cycle-regulated genes:

DNA replication:

POL1, POL2	pol1 and cdc20	DNA polymerases α and ϵ
RFA1	ssb1	Single-stranded DNA-binding protein
CDC6	cdc18	Regulator of DNA replication initiation
MRC1	mrc1	DNA replication checkpoint protein
RNR1	cdc22	Ribonucleotide reductase
SMC3, MCD1	psm3 and rad21	Cohesins
HTZ1	pht1	Histone variant
8 histone genes	9 histone genes	Histones H2A, H2B, H3, and H4

Others:

mik1	SWE1	Kinase inhibiting cyclin-dependent kinase
cig2	CLB1-CLB6	B-type cyclins
msh6	MSH6	Mismatch-repair protein
rhp51	RAD51	DNA repair protein

Human and fission yeast only:

cdc2 kinase and cdc25 phosphatase

Human and budding yeast only:

MCM complex DNA replication genes

Major Conclusions

- 4 major waves of transcription,
 3 clusters concentrated in M-S phases (~30% of cell cycle)
- ~400 periodic genes (8% of genome), 136 of which are of high amplitude; less periodic transcription than *S. cerevisiae*
- Conserved transcription factors but differences in regulatory circuits between fission and budding yeasts: rewiring during evolution to accommodate differences in cell cycle phases
- Periodic transcription not necessarily conserved, but core set of universally regulated genes with basic functions in cell-cycle progression

Expression Profiling During Sexual Differentiation:



Synchronized meiotic cultures

Transcription factor mutants and overexpressors

Dissect global gene expression program and its regulation



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 atf21/atf31 during meiotic differentiation:



atf21

wt

atf31





Genes regulated by Atf21/31:





~55% of late genes are *atf21/31* dependent

Regulatory Network During Meiosis



Inactivation of Stell transcription by Rep1



Nitrogen starvation:

Induced transcripts encode proteins with less nitrogen

-N genes

control: early genes

ecdf(y)



Content of nitrogen-rich amino acids

Comparison with meiotic transcriptome of budding yeast:



Genes in common:

- B-cyclins
- polo, flp1
- APC+ regulators
- recombination
- chrom. cohesion

Meiosis and sporulation similar in the two yeasts, but:

- small overlap in transcriptomes
- regulatory mechanisms not conserved

primitive meiosis in common ancestor, regulatory mechanisms evolving later?

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



<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:



Different regulation of stress response genes in two yeasts:

Budding Yeast

Fission Yeast



core stress response genes







Increasing cell density and stress gene expression:



Increasing cell density and stress gene expression:



Growth media influence stress gene expression:



Adversity has the effect of eliciting talents, which in prosperous circumstances would have lain dormant.

Horace

Expression profiling:

direct vs indirect effects? functional role

ChIP-chip:

- chromosome 'tiling' array of all intergenic regions
- immunoprecipitate DNA bd. protein,
 use bound DNA as target for hybridization
- map protein binding sites globally along chromosomes

Integrate expression and ChIP-chip data

direct

functional?

divergent genes?

Even if you are not equipped to do microarrays yourself, you can make good use of available data

It's out there...

Microarrays and I?

Examples:

Genes expressed at stage of interest: Toth et al. (2000) Cell 103:1155

Genes co-expressed with genes of interest: Colman-Lerner et al. (2001) Cell 107:739

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Thank you/

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