

Cold Adaptation in Budding Yeast

Schade, B., Jansen, G., Whiteway, M., Entian, K. D., & Thomas, D. Y. (2004). Cold adaptation in budding yeast. *Molecular biology of the cell*, 15(12), 5492-5502.

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Outline

- **Unicellular organisms respond to stress, little is known about cell response to cold shock.**
- **Cold shock affects transcriptional responses in *Saccharomyces cerevisiae*.**
- **Two distinct expression patterns are defined as an early (ECR) and late (LCR) cold response.**
- **Study suggests environmental stress response (ESR) is activated during the late cold response (LCR).**
- **Significant glycogen and trehalose accumulation and induction of transcriptional activators Msn2p and Msn4p occur during the LCR.**
- **ECR did not show markers of the ESR.**
- **Similar early up regulation responses observed between Schade et. al and wt genes after cold shock**

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Yeast cells respond to environmental changes

- **Genes are induced or repressed due to changes in the environment.**
- **Environmental Stress Response (ESR) is a function of transcription factors Msn2p and Msn4p that bind to stress response elements (STREs).**
- **Little is known about yeast growth and survival at low temperatures.**
- **Cold temperatures induce physical and biochemical changes, such as decreased membrane fluidity and membrane transport.**

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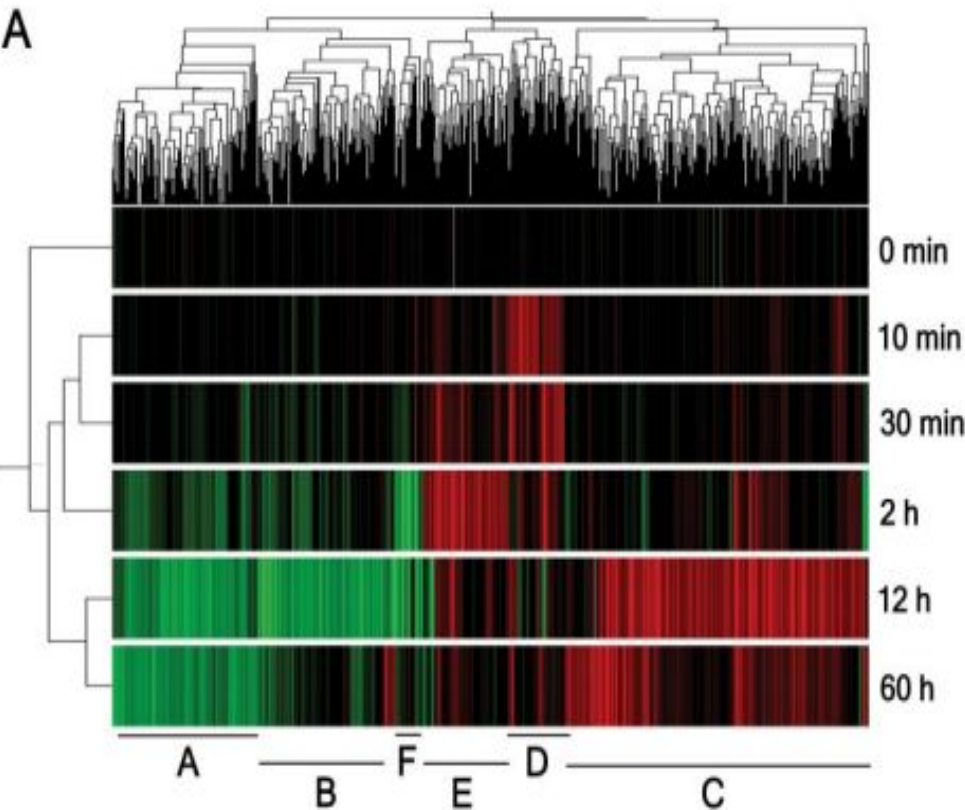
Cold shock in *S. cerevisiae* wild type and Δ msn2, Δ msn4 strains

- **Diploid strains were used to perform cold shock and DNA microarrays.**
- **Samples collected at 0m, 10m, 30m, 120m, 12 hours, and 60 hours.**
- **Glucose and trehalose concentrations in the yeast cells were determined for each sample.**
- **CS response was compared to various other stress responses**
- **Study compared experimental data to Gasch *et al.* (2000) and Sahara *et al.* (2002) data.**

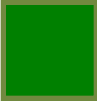
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6 profiles defined by similar expression patterns



- 634 genes examined
- Cold shock only:
 - 30°C to 10°C
- Clusters D, E are ECR
- Clusters A, B, C are LCR
- Trees show similarity of expression patterns at timepoints

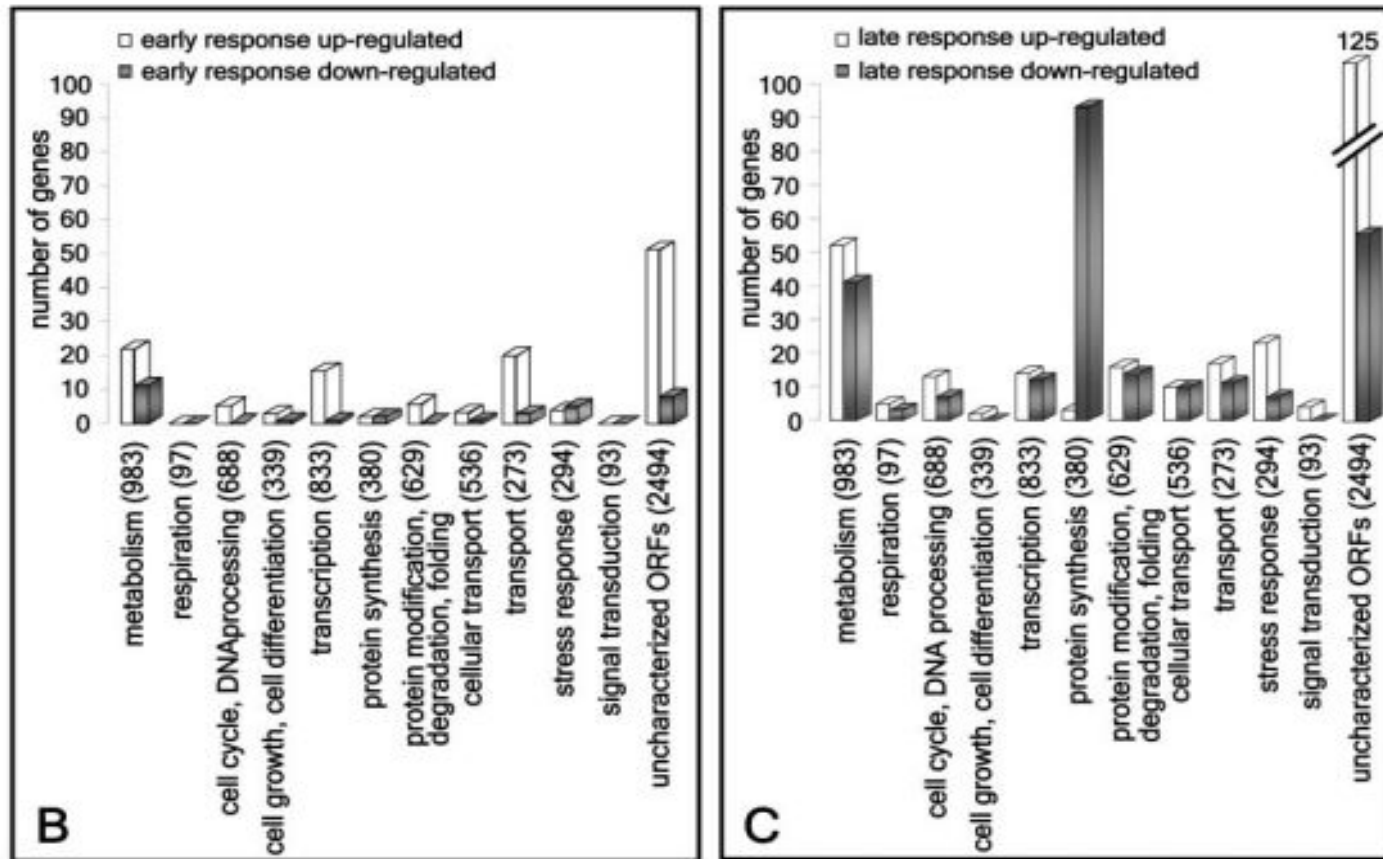
 = down regulated genes

 = up regulated genes

Outline

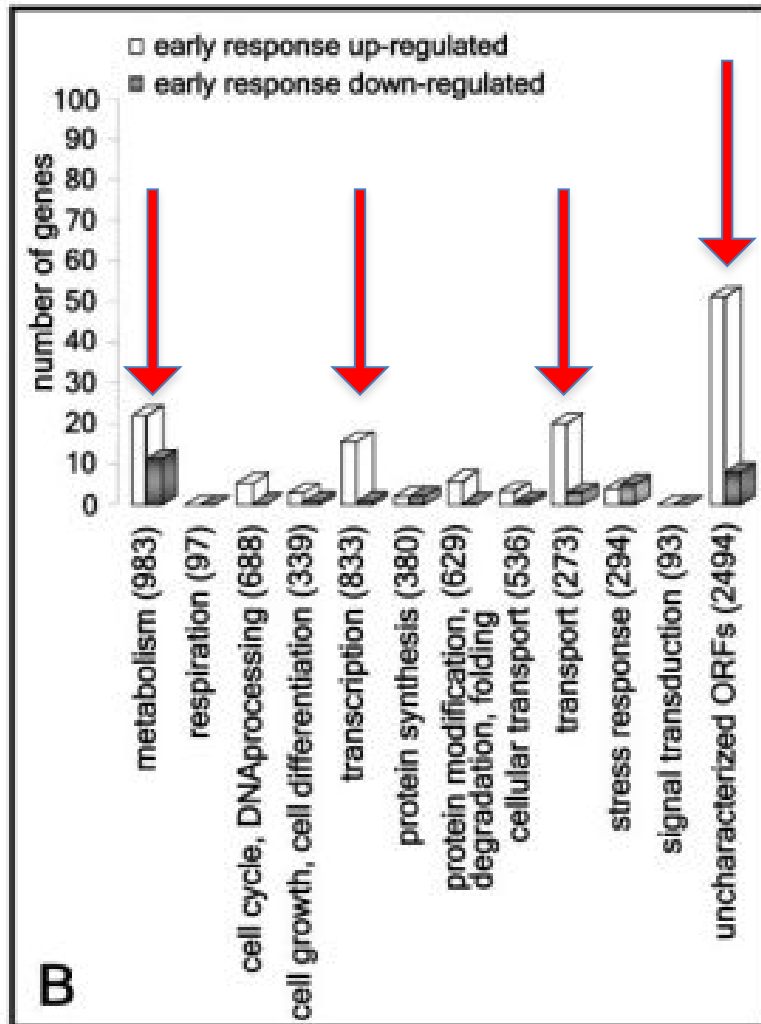
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Similarities between different gene expression patterns and exposure to cold



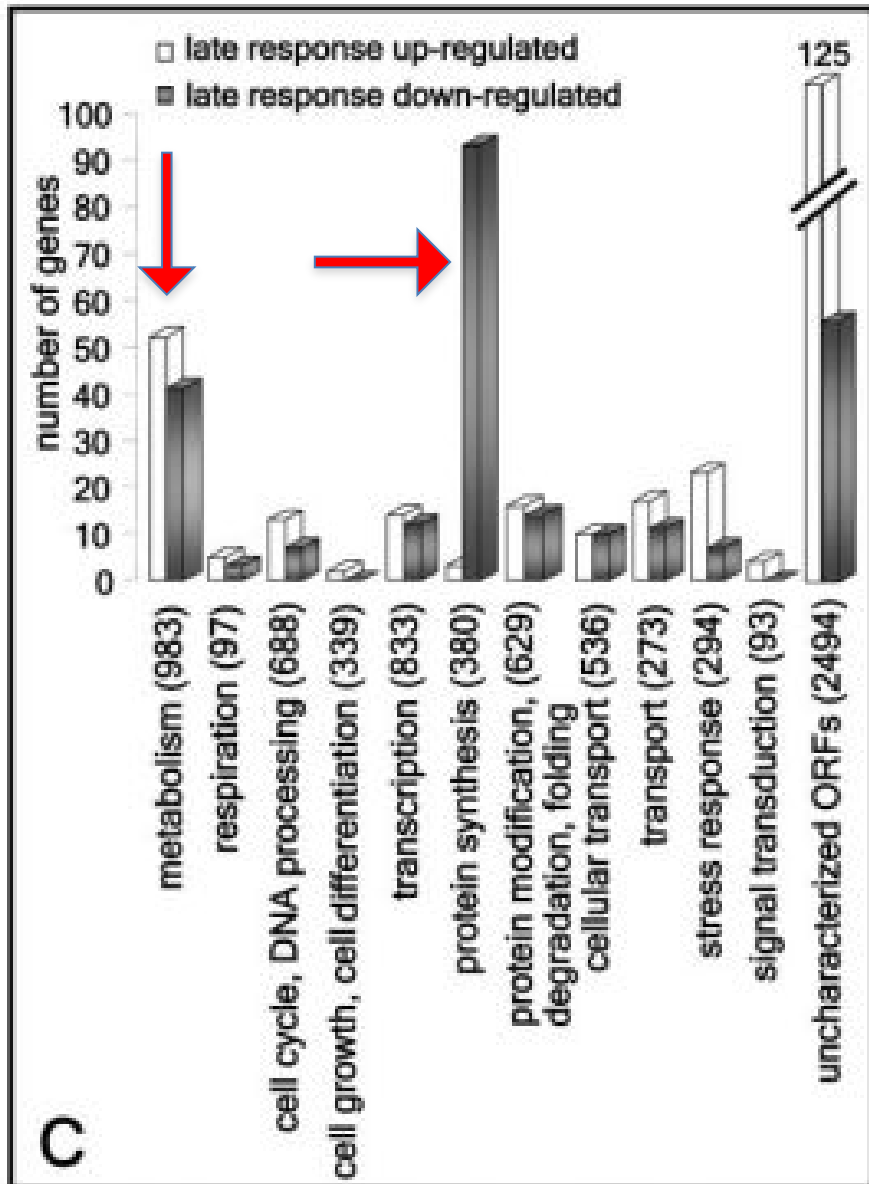
- Regulation responses in ECR and LCR

ECR demonstrates up-regulation of uncharacterized ORFs, metabolism, transcription, and transport



- Transcription genes included the RNA helicase genes, the RNA processing genes, and the RNA polymerase subunit gene.
- Genes involved in lipid metabolism involved in membrane fluidity were also affected (ex: OLE1).
- Expression of 32 genes were reduced by at least twofold in the first 2 hours.

Genes in LCR associated with carbohydrate metabolism



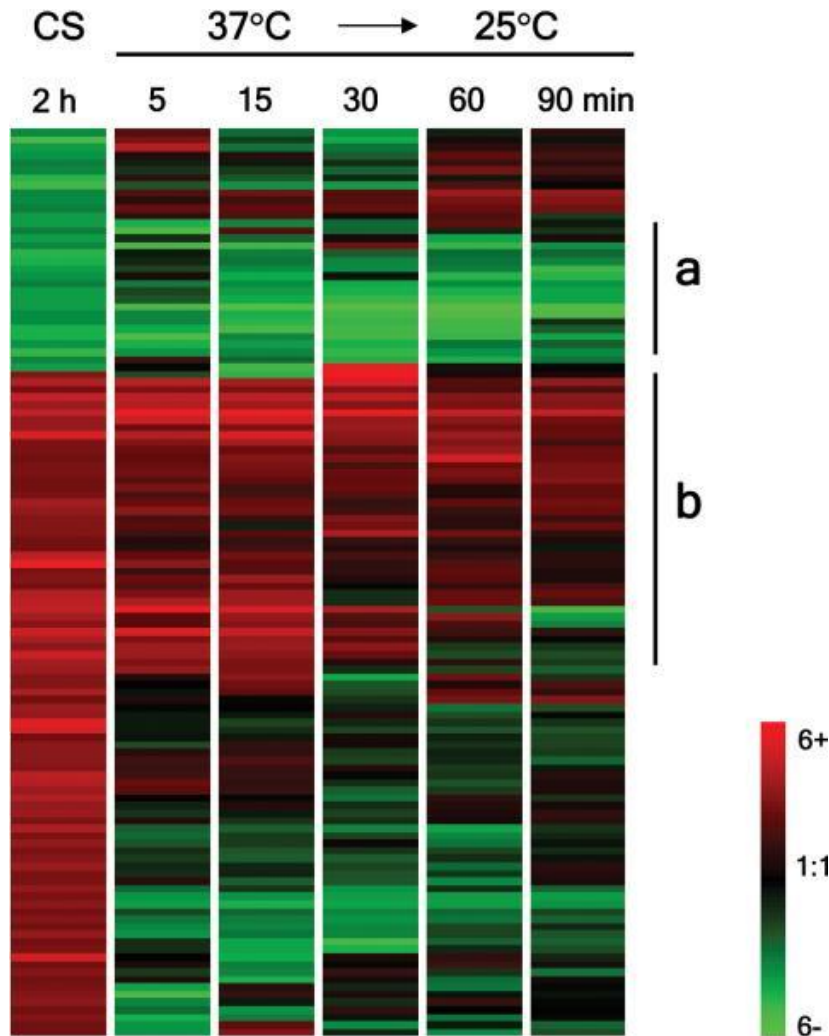
280 LCR genes were induced at 12 and/or 60 hours.

- Genes identified are involved in glycolysis, glycogen metabolism, and trehalose metabolism.
- Another set of induced LCR genes encode for heat shock proteins.

256 LCR genes were repressed.

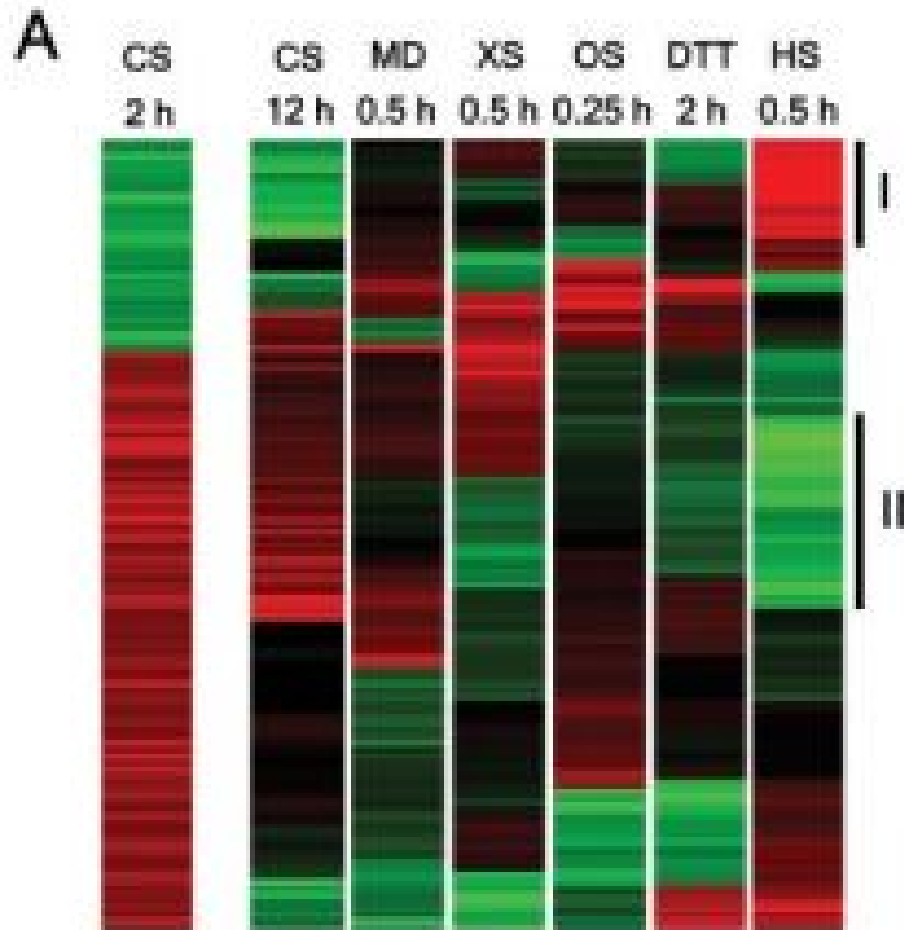
- Genes identified are involved in protein synthesis, nucleotide biosynthesis, protein modification and vesicle transport.

Temperature downshift yields similar response to early cold shock response



- 47% of induced early cold shock genes were induced by the temperature downshift (b).
- A large amount of down regulated genes in early cold shock were also repressed in the temperature downshift (a).

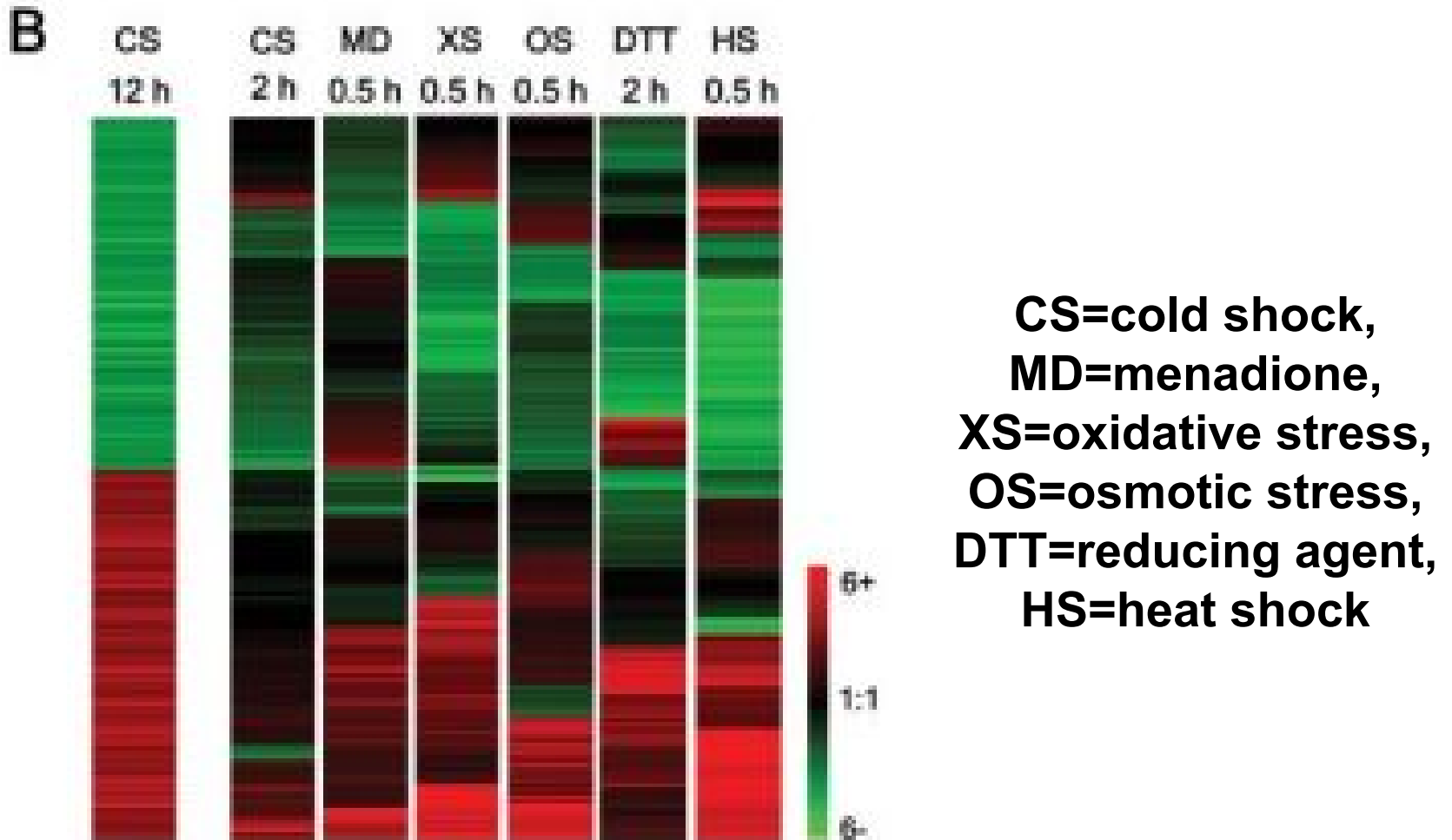
ECR genes showed reciprocal transcriptional behavior in comparison to other stress stimuli



CS=cold shock, MD=menadione,
XS=oxidative stress, OS=osmotic
stress, DTT=reducing agent, HS=heat
shock

- Comparison of ECR genes (CS 2 hours) to the responses to other stimuli.
- Half of repressed ECR genes were induced in heat shock.
- 40% of induced ECR genes were repressed after 0.5 hours of heat shock.
- (Gasch *et al.*, 2000) tried to show a common environmental stress response

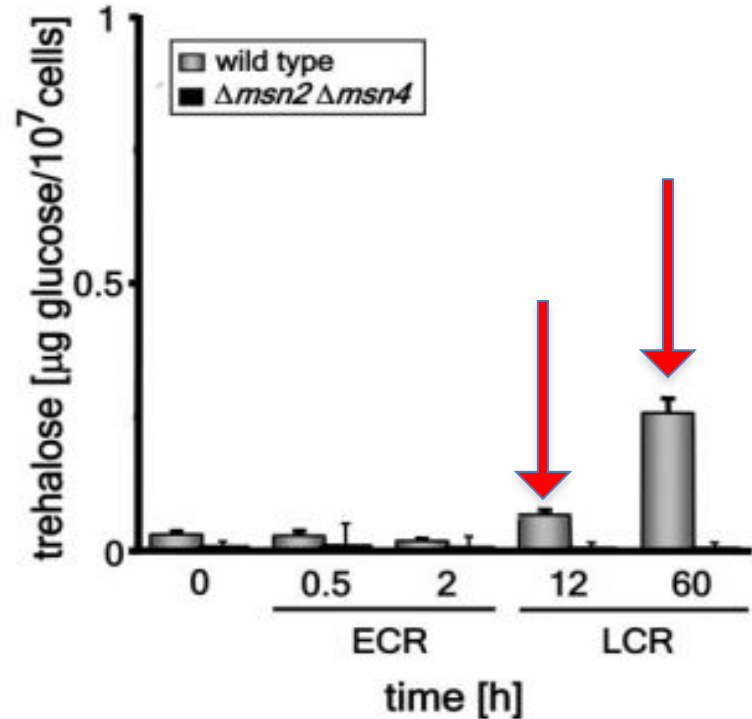
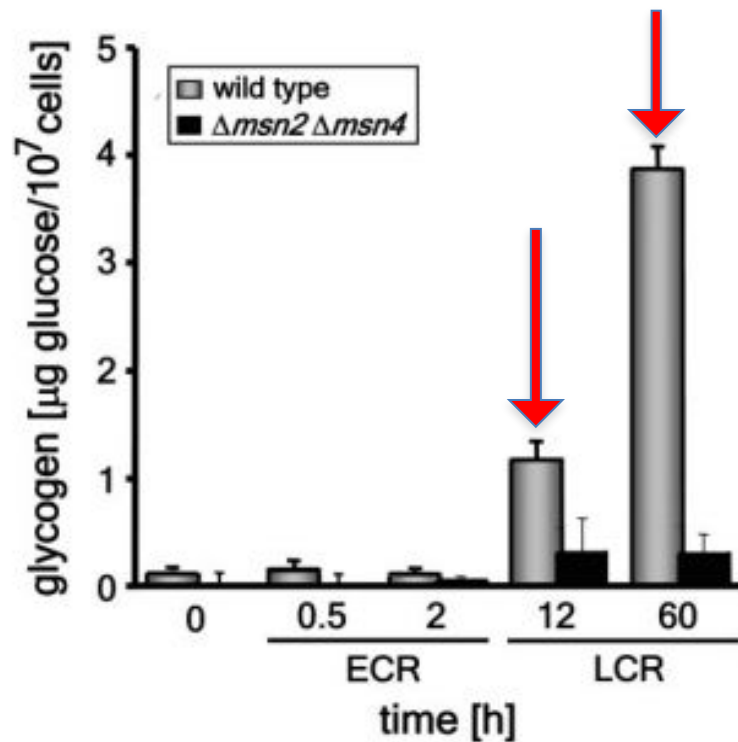
LCR showed similar transcriptional responses as other stimuli



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Glycogen and trehalose production are dependent on transcription factors msn2 and msn4

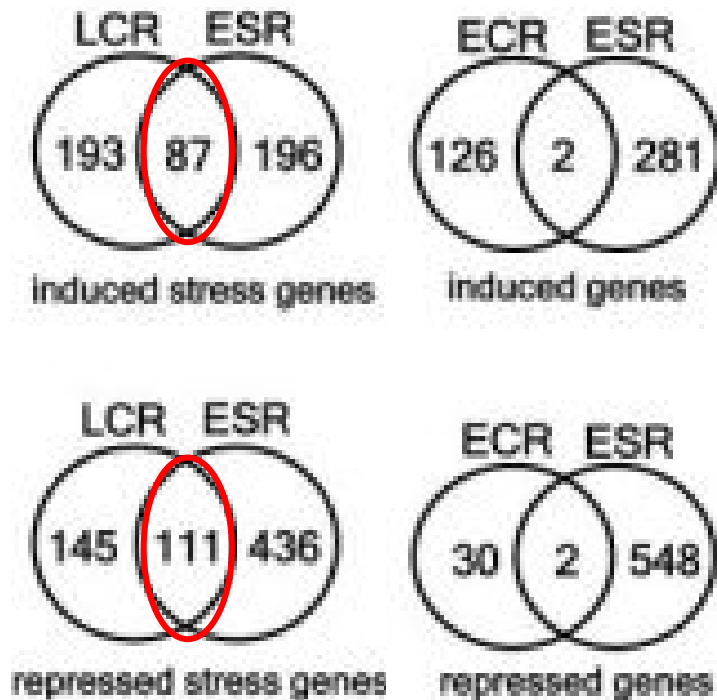


- Increase in glycogen and trehalose content observed after 12 hours (LCR).
- Mutant strains lacking Msn2p and Msn4p lose ability to produce glycogen and trehalose (carbohydrates)
- Carbohydrates are necessary to prevent freezing of water in the cell
- Ice crystals can poke holes in membranes and skew concentrations

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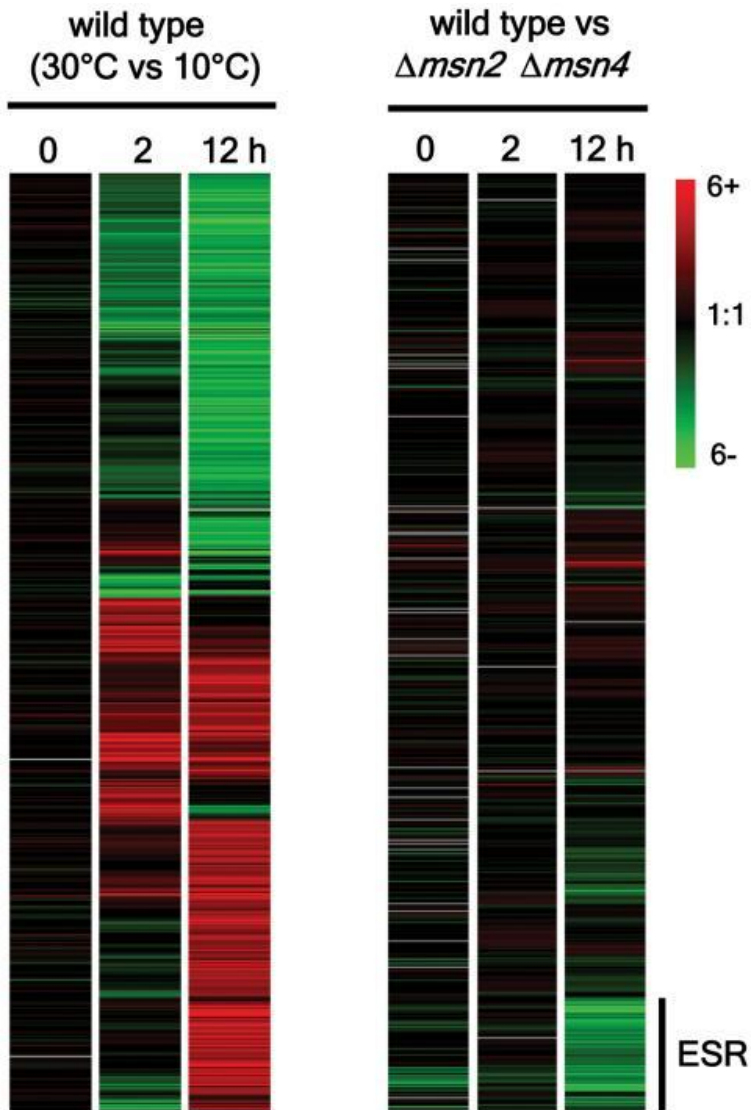
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Significant overlap between LCR and ESR for induced and repressed genes



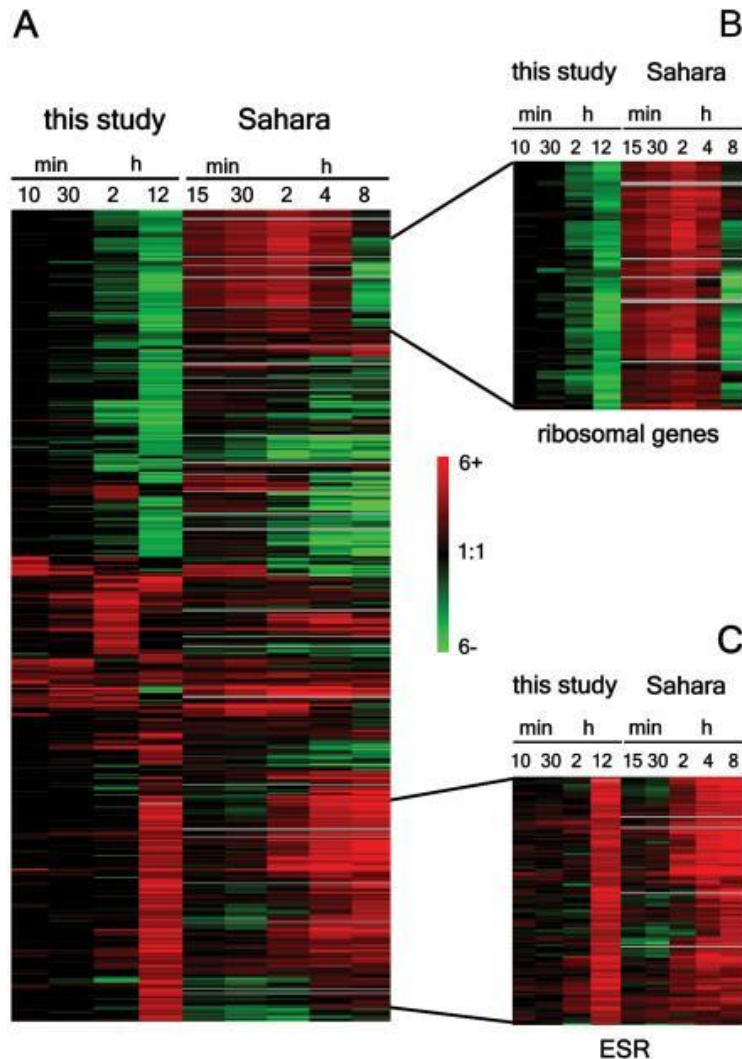
- Induced and repressed LCR and ECR genes compared to environmental stress response (ESR) genes (Gasch *et al.*, 2000).
- Induced and repressed LCR genes had a significant overlap of 87 and 111 genes with induced ESR genes.
- ECR and ESR genes did not have a significant overlap.
- Not enough genes studied

Msn2p and msn4p have little effect in early cold shock response



- Msn2p and msn4p are transcription factors that are required for 99 long cold shock response genes.
- There are other factors that control late cold shock response.

Comparison of Shade and Sahara *et al.* (2000) shows different results



- Comparison of 634 cold-responsive genes.
- Contradiction between Schade and Sahara *et al.* (2002). Induction versus repression of ribosomal genes.
- Consistency between the Schade and Sahara *et al.* (2002). Environmental stress response genes unregulated at times greater than 2 hours.

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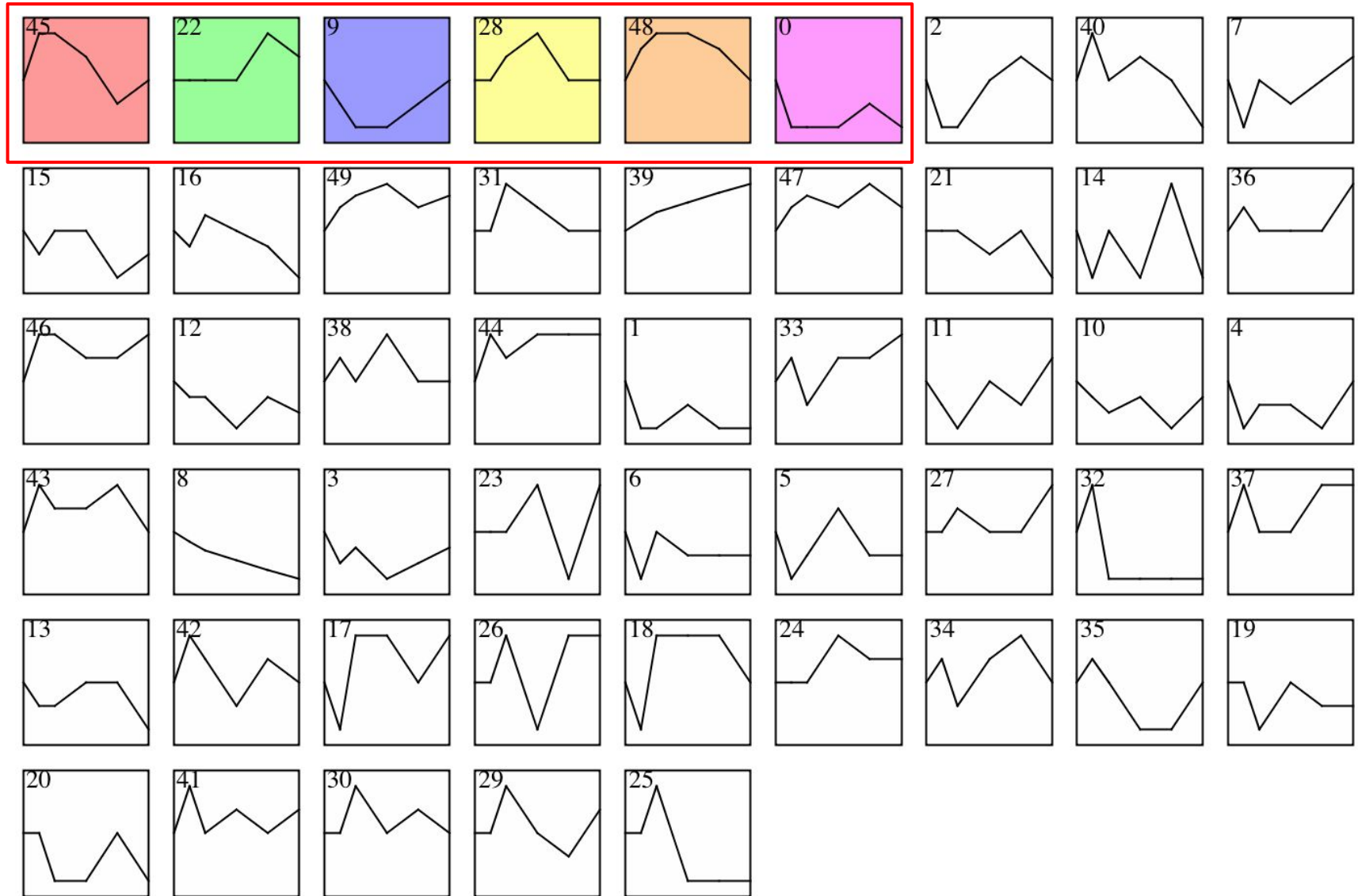
Shade and Dahlquist experiments differ in timepoints, temperature, and repeats

	Dahlquist	Schade
Strain	BY4741 (Wildtype-Diploid)	BY4743 (Wildtype-Haploid)
Timepoints	15min, 30min, 60min in cold 90min, 120min in recovery	10min, 30min, 2hour, 12hour, 60 hours in cold
Cold Temperature	13°C Cold Shock and Recovery	10°C Cold Shock
Medium	YPD (Rich Media)	YPD (Rich Media)
Replicates	4 for 15min, 60min 5 for 30min, 90min, 120min	3 for 10min, 30min, 60hour 2 for 2hour, 12hour

Wild type ANOVA test results

ANOVA	WT
$p < 0.05$	2600 (42%)
$p < 0.01$	1727 (28%)
$p < 0.001$	1015 (16.4%)
$p < 0.0001$	574 (9.3%)
B & H $p < 0.05$	302 (4.88%)
Bonferroni $p < 0.05$	1936 (31.3%)

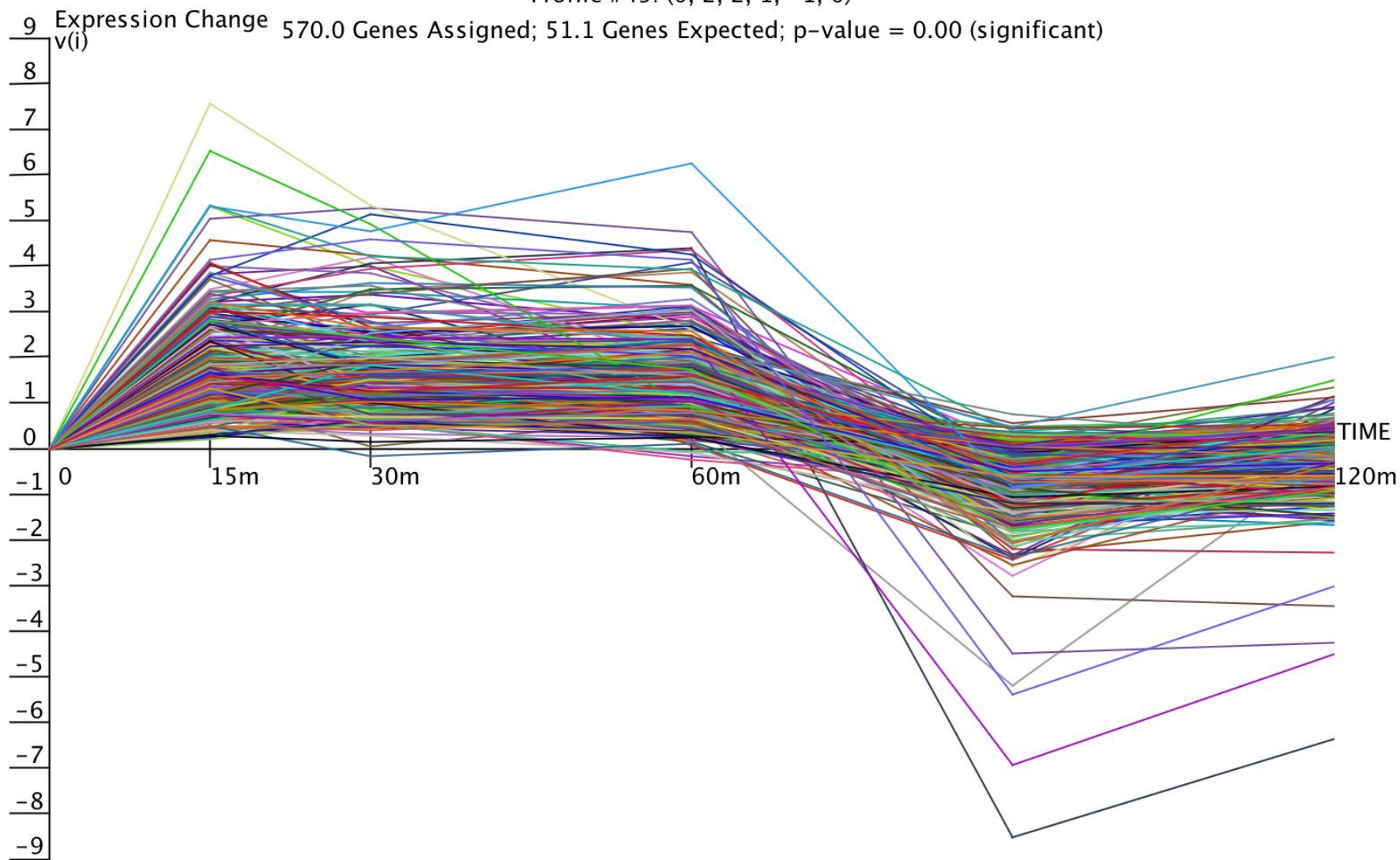
Significant Profiles # 45, 22, 9, 28, 48, and 0





Profile # 45 showed early gene up regulation, down regulation during recovery

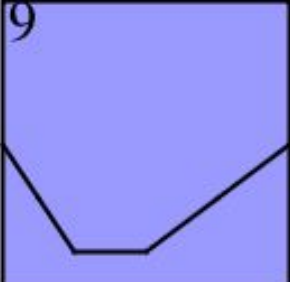
Profile #45: (0, 2, 2, 1, -1, 0)





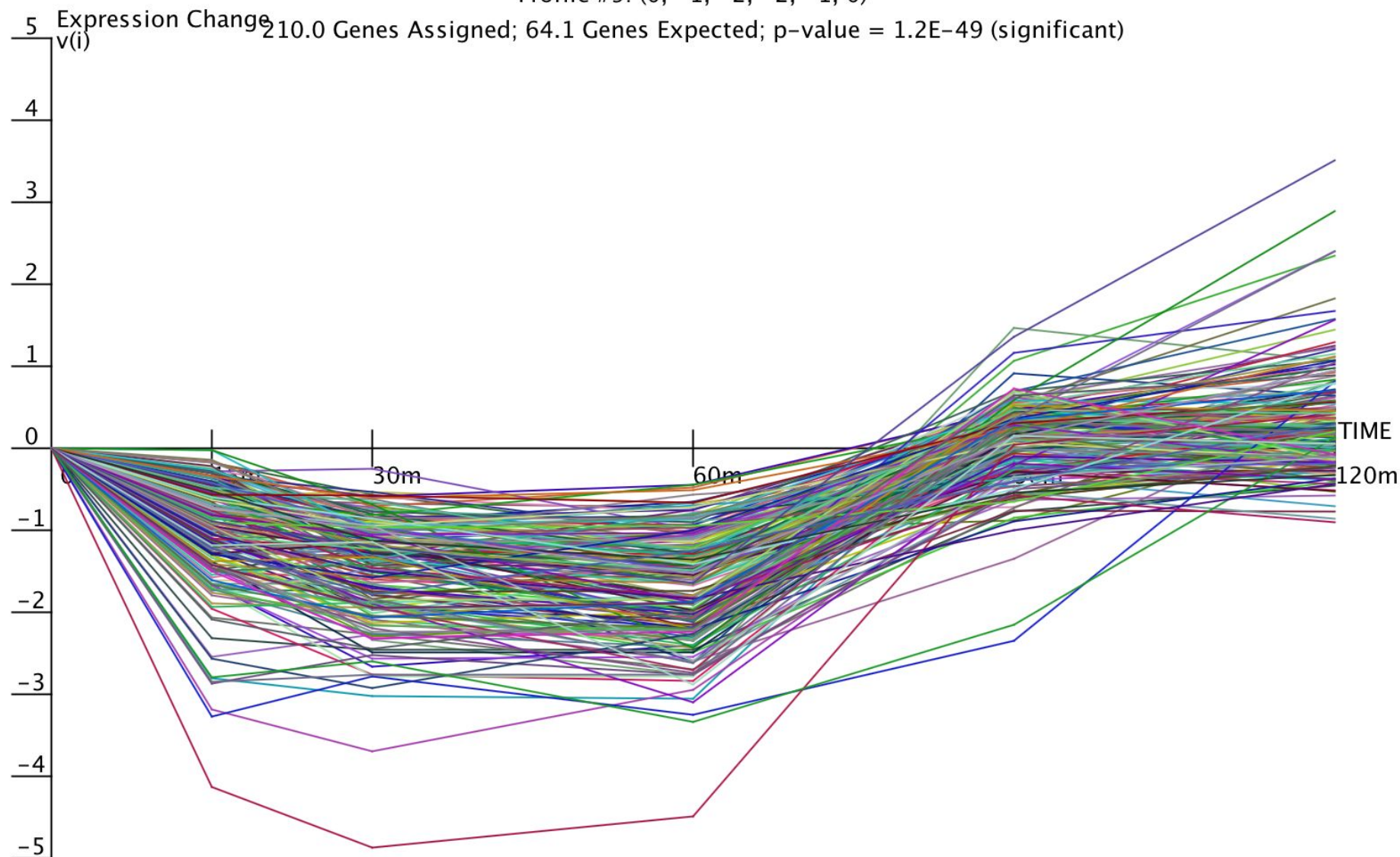
Summary statistics from GO list for Profile # 45

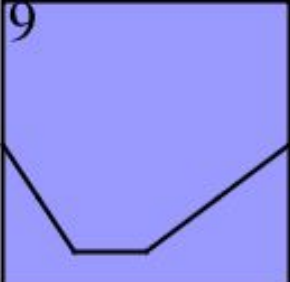
GO Term	Genes	Genes Assigned	Genes Expected	Genes Enriched	P-value	Corrected P-value	Fold
rRNA binding	33	23	9.4	11.6	9.6E-07	0.002	2.4
nuclear export	47	27	13.4	13.6	2.7E-05	0.016	2
Nitrogen compound metabolic process	840	286	240.2	45.8	3.00E-	0.006	1.2
RNA 3' end processing	17	13	4.9	8.1	5.50E-05	0.028	2.7
ribosomal subunit export from nucleus	22	16	6.3	9.7	2.10E-05	0.014	2.5
nuclear RNA surveillance	15	12	4.3	7.7	5.10E-05	0.028	2.8



Profile # 9 demonstrates early down regulation

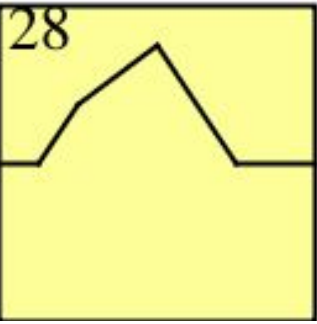
Profile #9: (0, -1, -2, -2, -1, 0)



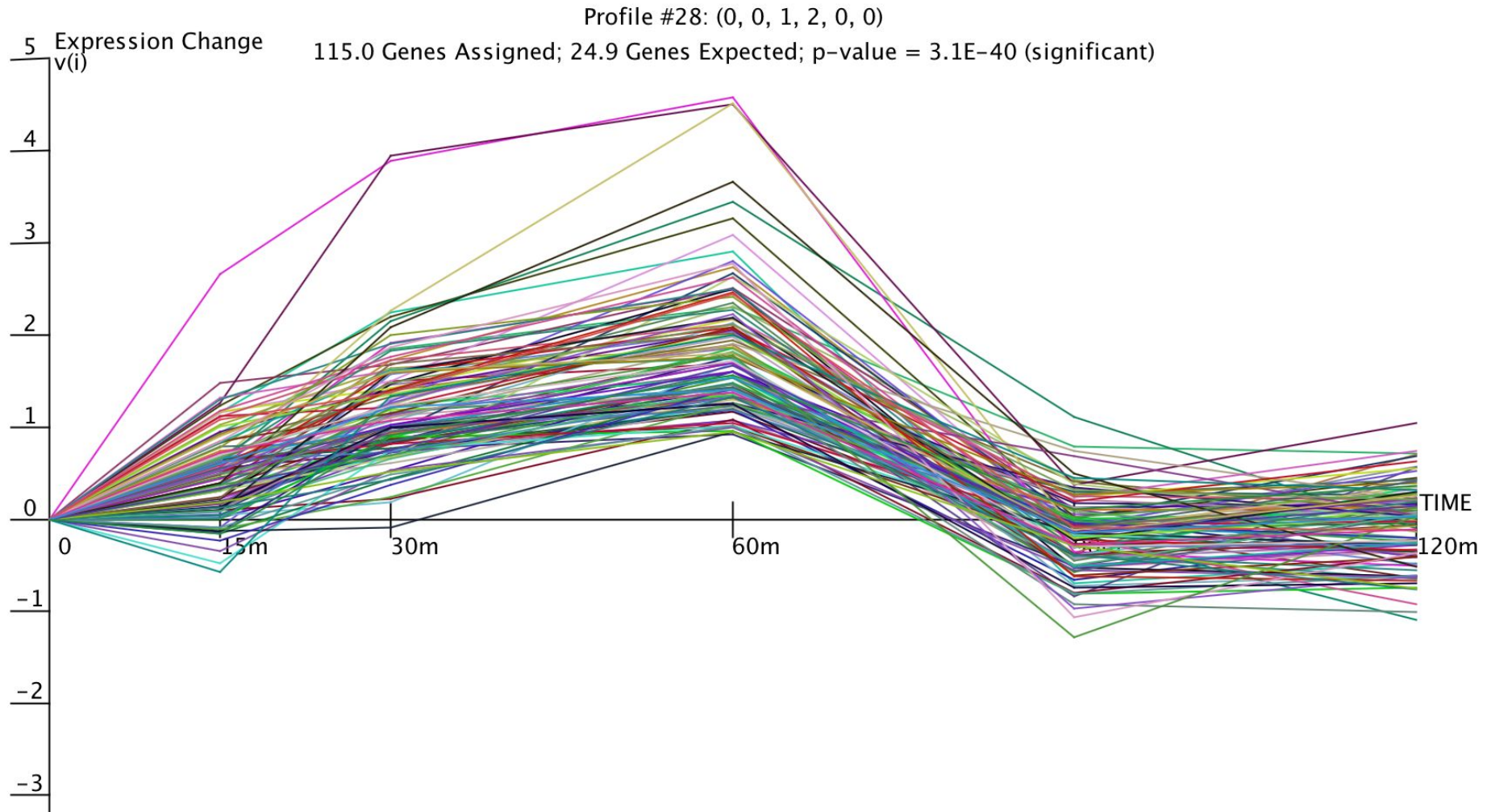


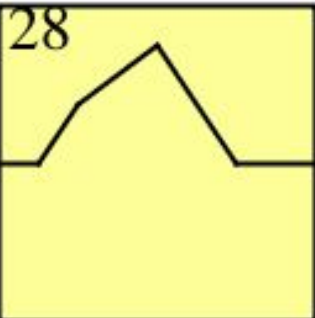
Summary statistics from GO list for Profile # 9

GO Term	Genes	Genes Assigned	Genes Expected	Genes Enriched	P-value	Corrected P-value	Fold
Cytoplasm	1380	182	145.4	36.6	5.60E-10	0.001	1.3
Vesicle	81	23	8.5	14.5	3.90E-06	0.001	2.7
Cytosol	265	50	27.9	22.1	7.80E-06	0.001	1.8
Golgi Apparatus	66	19	7	12	2.3E-05	0.012	2.7
Protein Localization	218	42	23	19	3.00E-05	0.014	1.8
Cytosolic Ribosome	33	12	3.5	8.5	6.60E-05	0.024	3.5



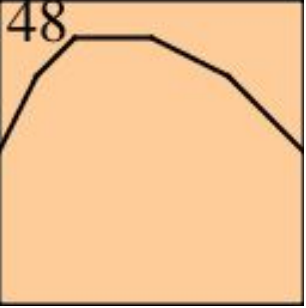
Profile # 28 demonstrates early up regulation



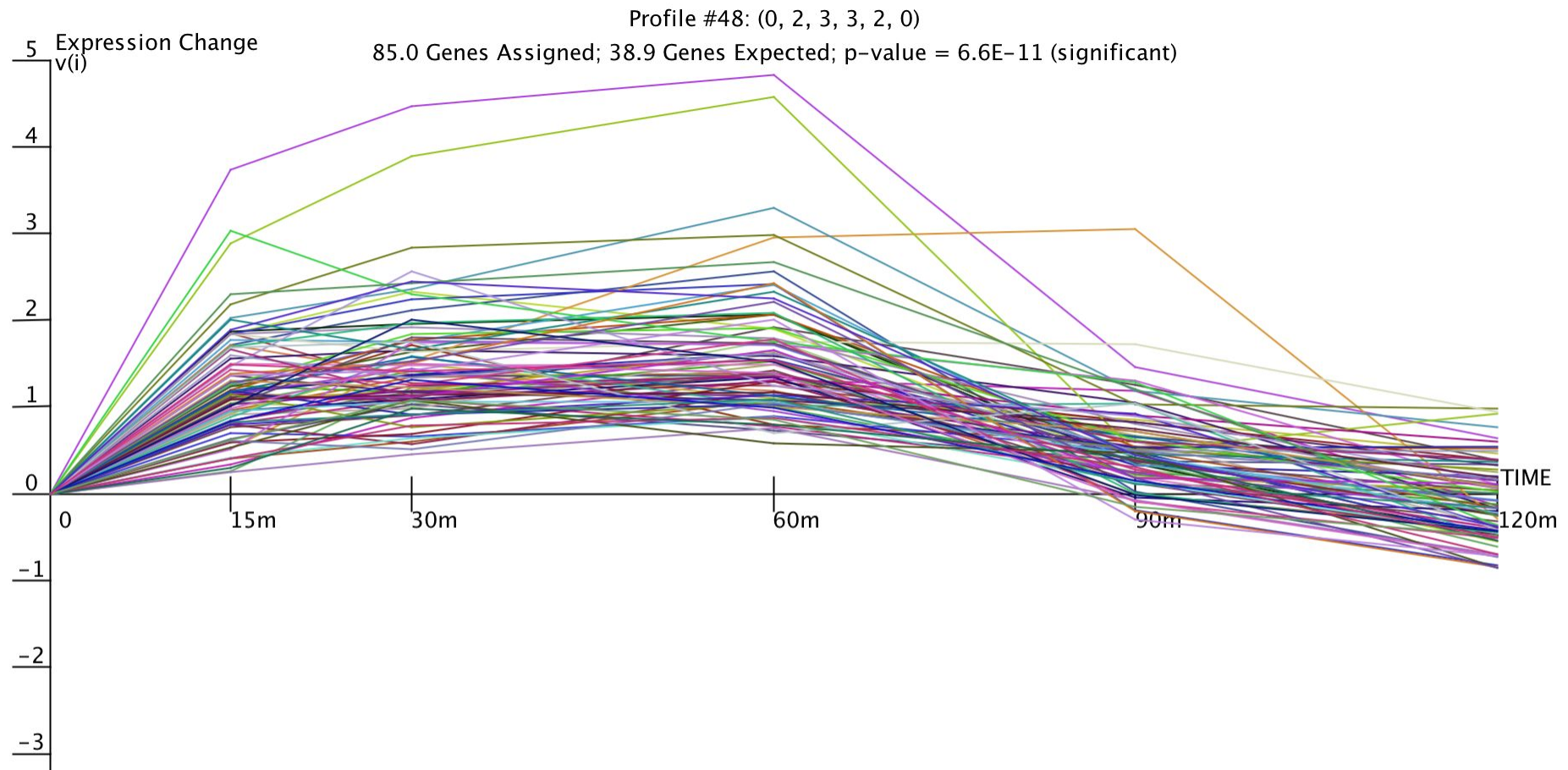


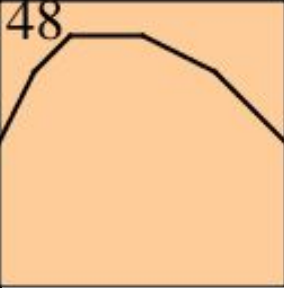
Summary statistics from GO list for Profile # 28

GO Term	Genes	Genes Assigned	Genes Expected	Genes Enriched	P-value	Corrected P-value	Fold
Cell Wall Organization	78	15	4.5	10.5	2.10E-05	0.002	3.3
ATP Binding	179	19	10.3	8.7	5.40E-03	0.704	1.8
Cellular Amino Acid Metabolic Process	98	17	5.7	11.3	2.40E-05	0.006	3
Double Strand Break Repair	34	5	2	3	.04	.99	2.5
Protein Phosphorylation	68	9	3.9	5.1	0.01	0.916	2.3
Small Molecule Biosynthetic Process	130	17	7.5	9.5	8.90E-04	0.23	2.3



Profile # 48 shows early up regulation





Summary Statistics from GO List for Profile # 48

GO Term	Genes	Genes Assigned	Genes Expected	Genes Enriched	P-value	Corrected P-value	Fold
transcription initiation from RNA polymerase II promoter	19	5	0.8	4.2	9.10E-4	0.176	6.2
DNA-templated transcription, initiation	24	5	1	4	2.80E-3	0.428	4.9
proteasome-mediated ubiquitin-dependent protein catabolic process	43	6	1.8	4.2	8.60E-3	0.712	3.3
RNA splicing, via transesterification reactions	34	5	1.5	3.5	0.01	0.824	3.4
regulation of cellular protein metabolic process	92	8	3.9	4.1	0.04	0.986	2
covalent chromatin modification	40	5	1.7	3.3	0.03	0.964	2.9

GO terms and gene expression show limited similarities between early up regulated and wt genes from (*Schade et. al 2004*)

- Profiles 28, 45, 48, and genes from (*Schade et. al*) show early up-regulation expression of metabolic processes after cold shock
- Profile 45 shows significant expression of export genes during early up-regulation, similar to the expression patterns in (*Schade et. al 2004*)
- Genes affected by cold shock were expected to show more similar expression patterns
 - More data collection and GO term analysis may show a stronger correlation

LCR genes associated with glycolysis, glycogen metabolism, and trehalose metabolism in (*Schade et. al 2004*)

- Data infers a similarity between LCR and Environmental Stress Response (ESR) in terms of Induced and repressed genes.
 - Could be due to organism's acknowledgment of cold as potential threat.
- LCR shared similar transcriptional responses with other stimuli responses
 - Implies genetically overarching response to variety of threat factors
- Similar early up regulation responses observed between (*Schade et. al*) and wt genes after cold shock

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Dr. Dahlquist

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Bioinformatics Lab, Fall 2016

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