A transcriptomic analysis of photomorphogenesis in *Arabidopsis Thaliana*

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Outline

- Introduction :- A. Thaliana as a model organism
- Photomorphogenesis
- A conservative estimate of differentially expressed genes
- A strategy for examining functional classification
- The picture of photomorphogenesis
- Where to go from here...

Why is *Arabidopsis thaliana* a good system ?

- Short lifetime - around 40 days.
- Large number of genes - ~25,500 (*Drosophila* around 11,000 genes)
- Compact Genome - 125 Mbases (non-coding regions ~30-50%)
- Large number of array experiments (65 Affymetrix data sets, with 10's of raw image files per data set at TAIR for example)
- A huge number of different strains - over half a million genotypes.

And why perhaps not so good....

- Not much Protein-Protein interaction data (Tandem Affinity Purification experiments on the way).

- The genomes of near neighbours have not been sequenced.

    - *A. lytra, C. rubella, B. rapa and T. halophilia* are planned or underway

- Not clear how representative genes are for other agriculturally relevant species

    - A. thaliana has a huge repertoire of Ubiquitination proteins.

Photomorphogenesis in *Arabidopsis thaliana*

- Before exposure to light, seeding grows via skotomorphogenesis after germination - slow root growth, no growth in shoot apical meristem or cotyledon.

- When exposed to light, cotyledon grows through simple reproduction (control).

- Meristem (stem cells) grow by differentiation.

- Meristem source of true leaves.

- Little understood about process.

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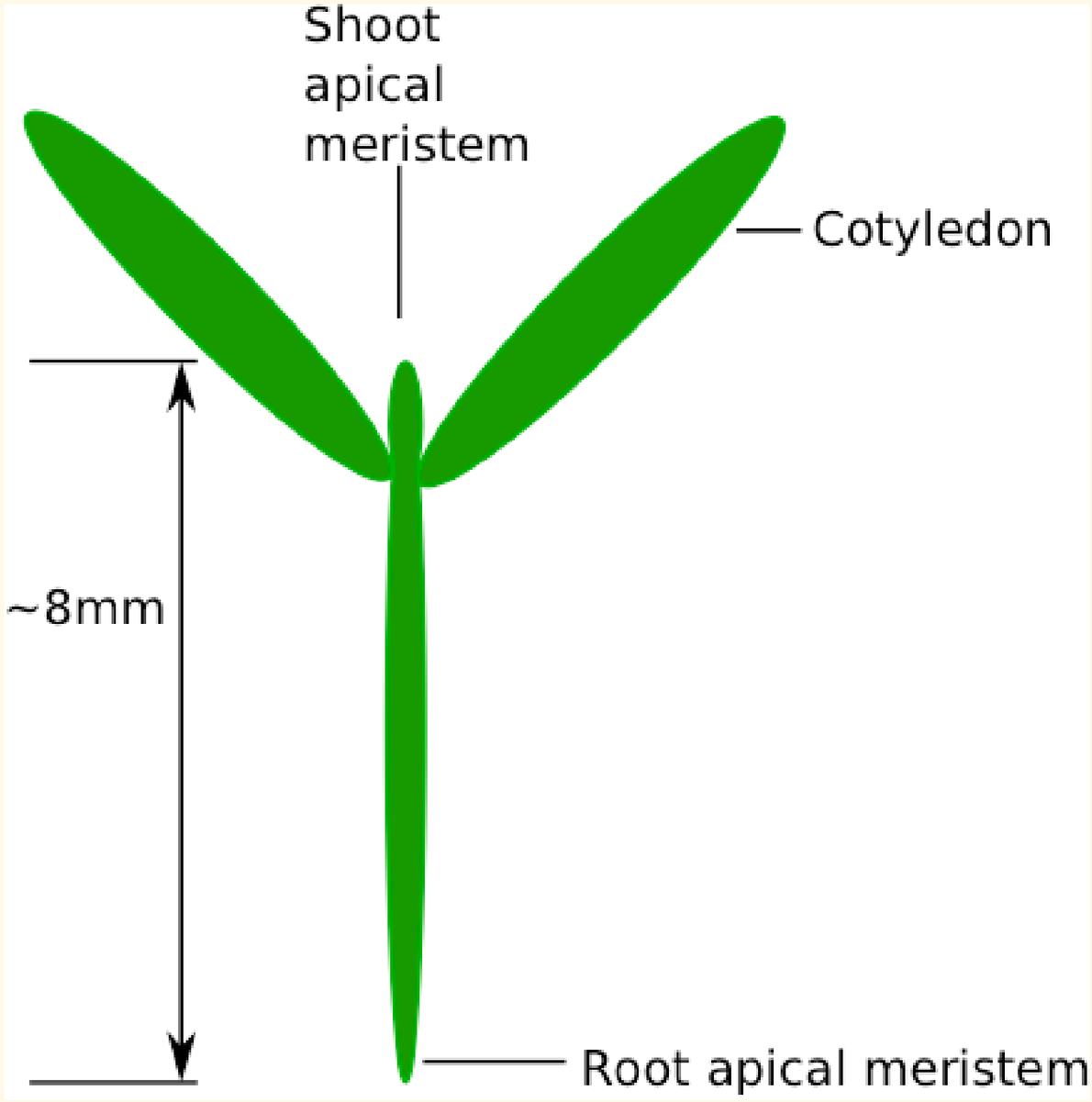


The data

- RNA material was gathered from the shoot apical meristem and cotyledon of Arabidopsis seedlings at
    - 0 hour (in darkness)
    - 1 and 6 hours (Cot and Mer with replicates)
    - 2, 24, 48 and 72 hours (Mer only)
- Samples hybridised with Affymetrix ATH1 GeneChip array.
- No amplification of RNA material !

Strategy

- Construct stringent test to determine genes which are clearly differentially expressed.

- Identify kinetic behaviour of different classes of differentially genes (i.e. try and find a time line of events).

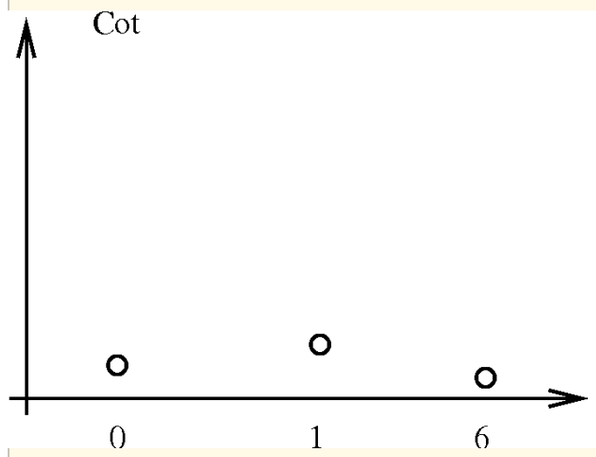
- Identify functional groupings of genes and then examine how all the genes in that functional grouping behave (i.e. including those that are not differentially expressed according to our strict criteria).

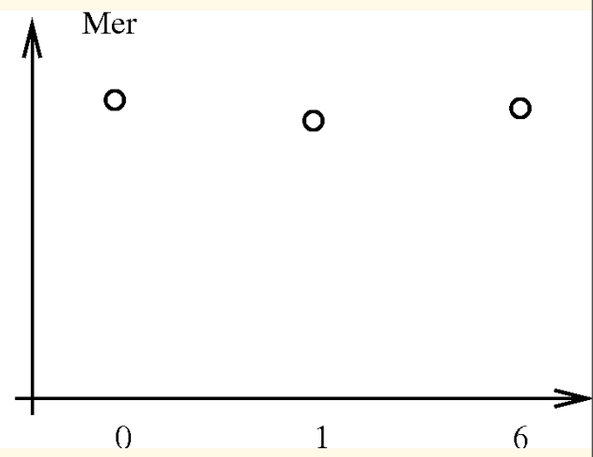
Finding differentially expressed genes

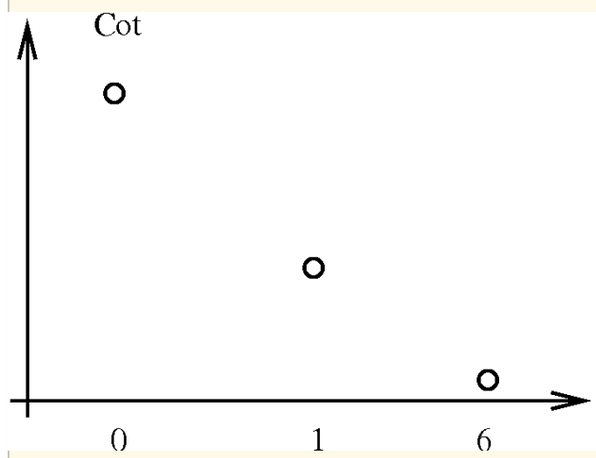
- Look at three different normalisations for Affymetrix data
    - GCRMA
    - MAS5
    - VSN
- Only consider genes that are differentially expressed in all three normalisations as being significant.

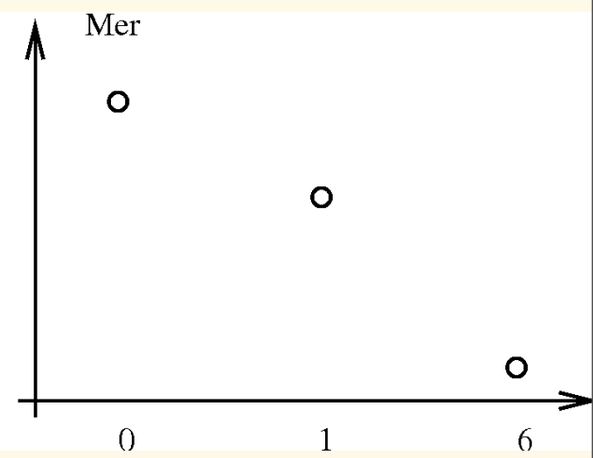
Test for significance

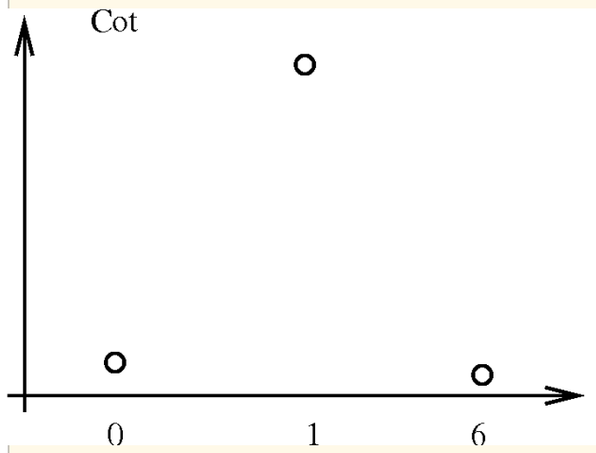
- Apply two-way ANOVA test. Look for significance with respect to
  - tissue
  - time
  - time and tissue
- Compute F-Ratio
- Only use data with two replicates (i.e. Cot and Mer at 0, 1 and 6 hours)

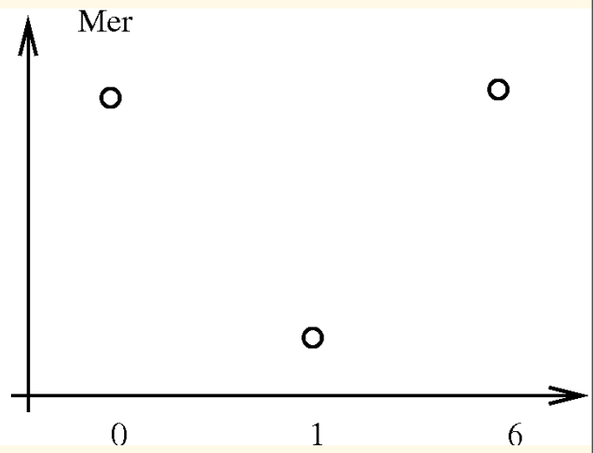












Finite sample size :- bootstrapping

- 2 replicates for the ANOVA data set.

- Cannot trust a p-value from such data !

- Solution :- create a large set of artificial data by randomly selecting expression values from all of the data.

- Compute histogram of resulting F-values for ANOVA test to determine a p-value.

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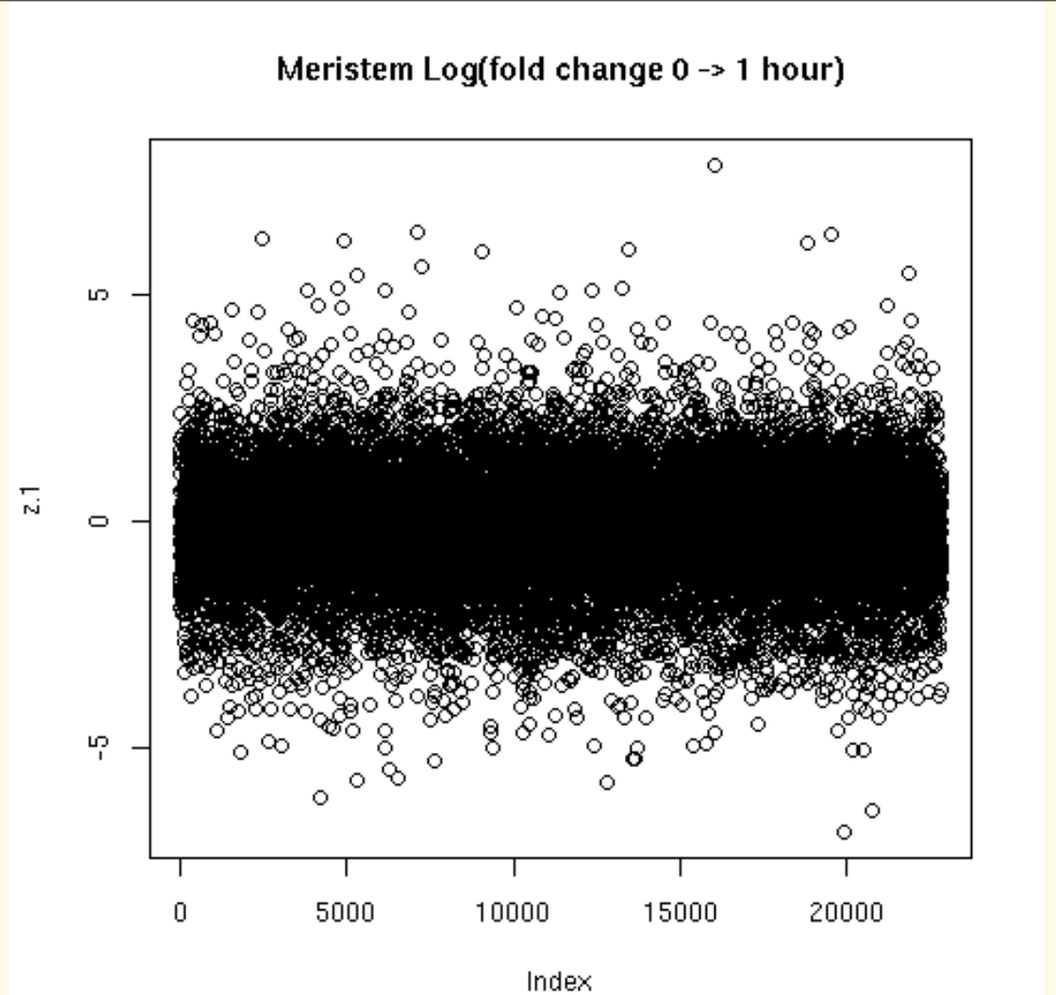

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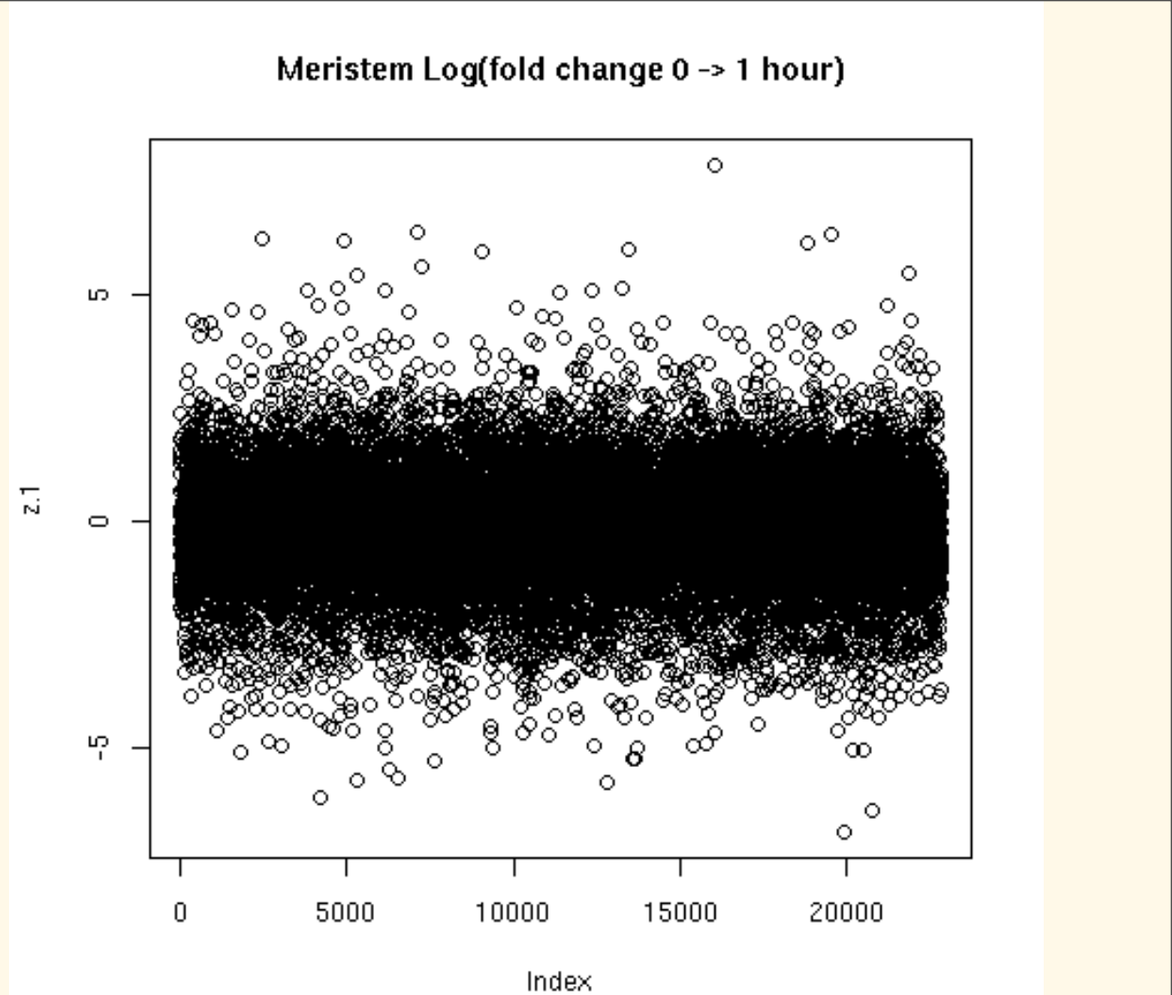
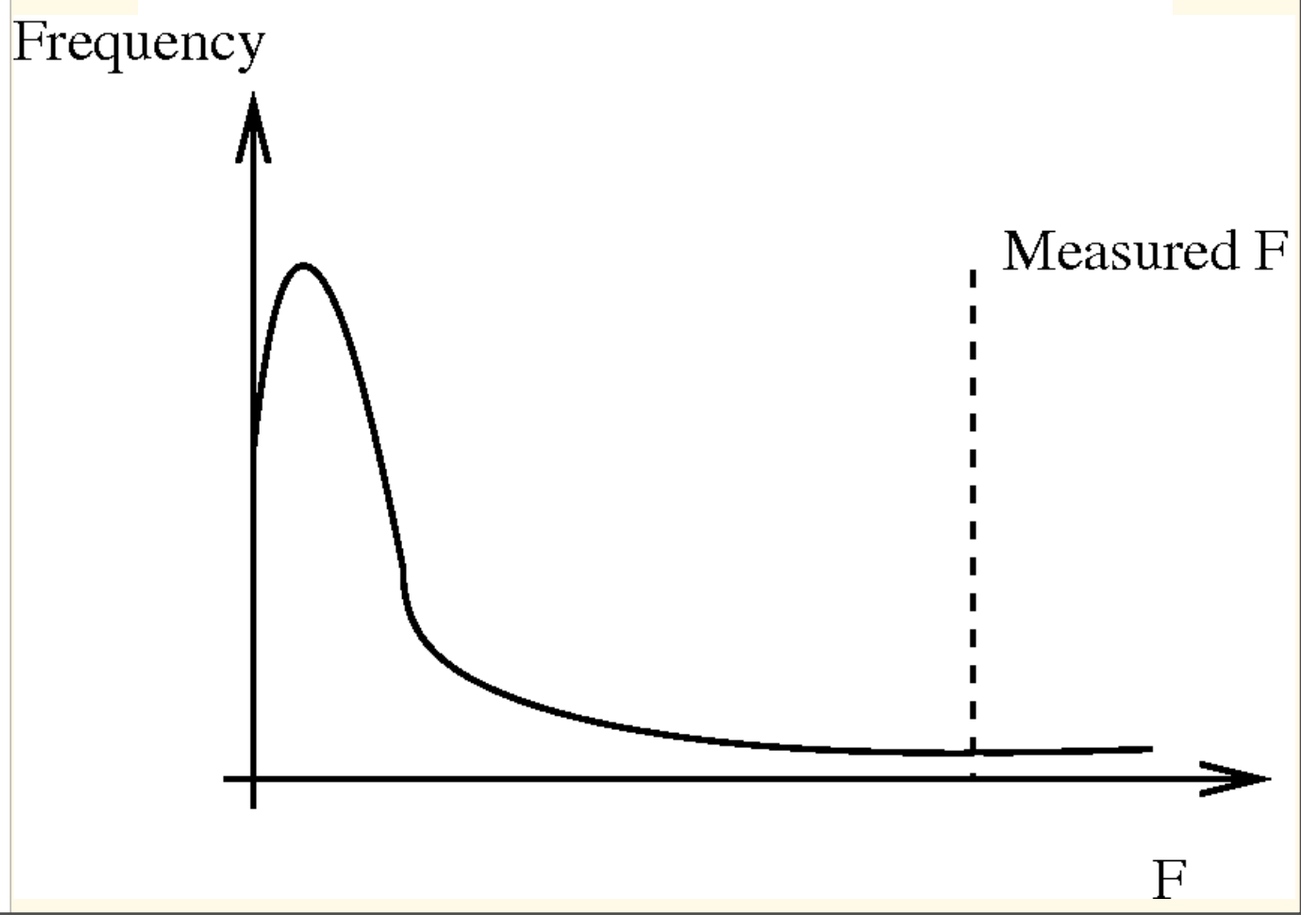
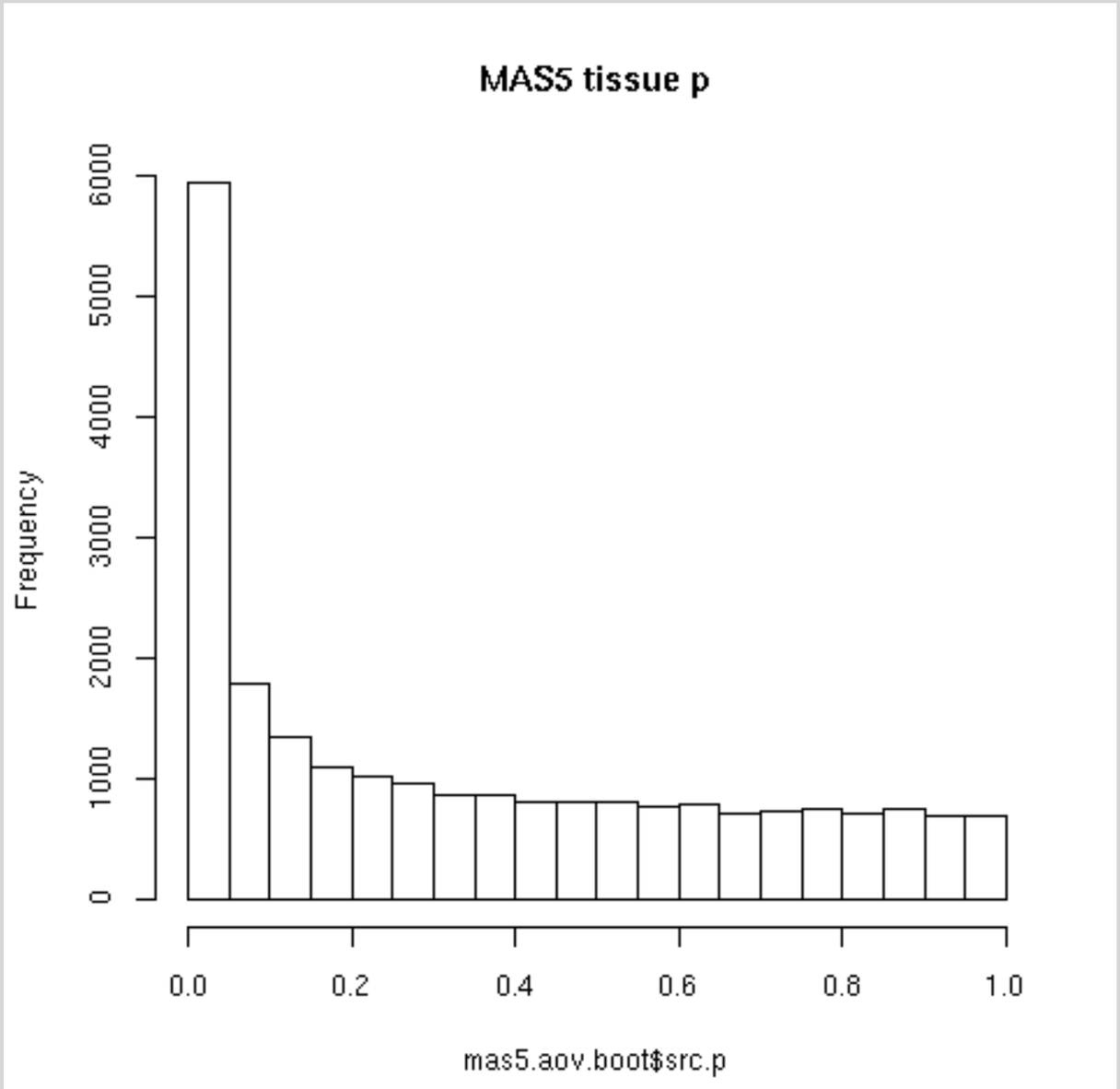




False Detection Rate

- Bonferroni Correction is very conservative.
- Estimate FDR by plotting a histogram of the p-values.
- Fix FDR to 5% and set p-value.
- Important step : employ Present/ Absent filter in MAS5 to filter out genes. Substantial improvement in results.
- Final step : remove all genes with fold change less than 2



Initial Results

- Selected 5,620 genes (out of 22,810).
    - (Very conservatively) 1/4 of the transcriptome is differentially expressed during photomorphogenesis.
- Majority selected through time variation (2/3 time, 1/3 tissue).
- Very small number selected using time-tissue variation (10).

Functional Classification :- it should be easy....

- Many genes in Arabidopsis have some kind of functional annotation.
- Use Gene Ontology to give a structured functional annotation.
- Enumerate numbers of genes for a given annotation.
- Compute probability of over or under-representation using hyper-geometric distribution.

Functional Classification :- but it isn't....

- Gene Ontology are useful but far too specific at its lowest nodes - False Discovery Rate calculation.

- Initially interested in general picture, what is the highest level annotation ?

- GO slim should cover more general cases, however annotations of genes can have multiple parents, e.g. a gene with kinase function and binds to DNA will sit in both classes.

- Ultimately, we developed our own general functional annotation.

- Lesson :- GO has a huge amount of information, but when looking at the big picture, you need to make the decisions !

Gene Model: AT5G60890.1 [Help]

Update History	AT5G60890.1 replaces AT5G60890.1 on 2004-02-23
Date last modified	2007-04-17
Name	AT5G60890.1
Name Type	orf
Gene Model Type	protein_coding
TAIR Accession	Gene:3441888
Description	Myb-like transcription factor that modulates expression of ASA1, a key point of control in the tryptophan pathway; mutant has deregulated expression of ASA1 in dominant allele. Loss of function allele suggests ATR1 also functions at a control point for regulating indole glucosinolate homeostasis.
Chromosome	5
Locus	AT5G60890 (Note: use this locus link to see associated gene models, markers and ESTs).

Gene Alias

name	type
MSL3_10	orf
MSL3.10	orf
ATMYB34	symbol
ATR1	symbol
MYB DOMAIN PROTEIN 34	full_name

Annotations

Category	Relationship Type	Keyword
GO Biological Process	involved in	response to salt stress, response to abscisic acid stimulus, response to gibberellin stimulus, response to jasmonic acid stimulus, response to salicylic acid stimulus
GO Cellular Component	located in	nucleus
GO Molecular Function	functions in	DNA binding
	has	kinase activity, transcription factor activity

Annotation Detail

Protein Data

name	Length(aa)	molecular weight	isoelectric point	domains(# of domains)
AT5G60890.1	295	32743.0	4.9866	Myb, DNA-binding;Molecular Function: DNA binding (:IPR001005(7)

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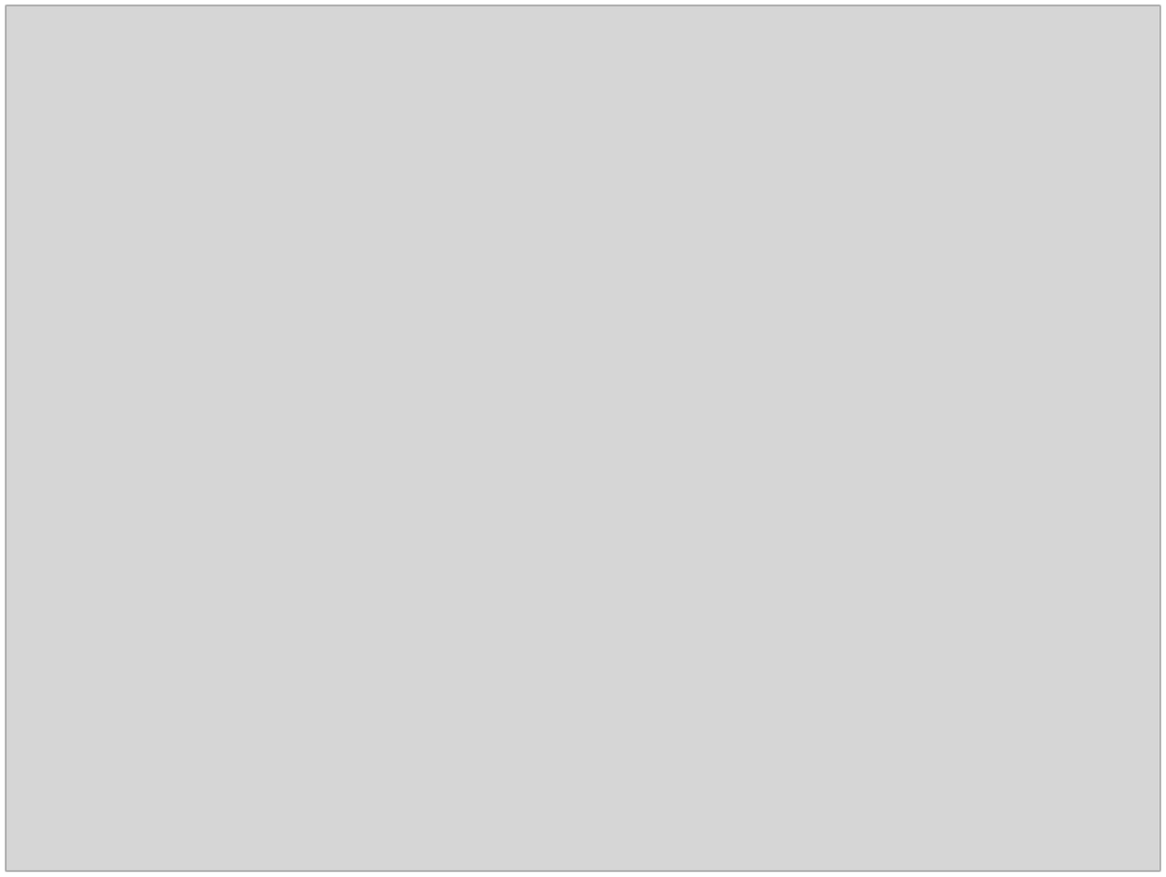
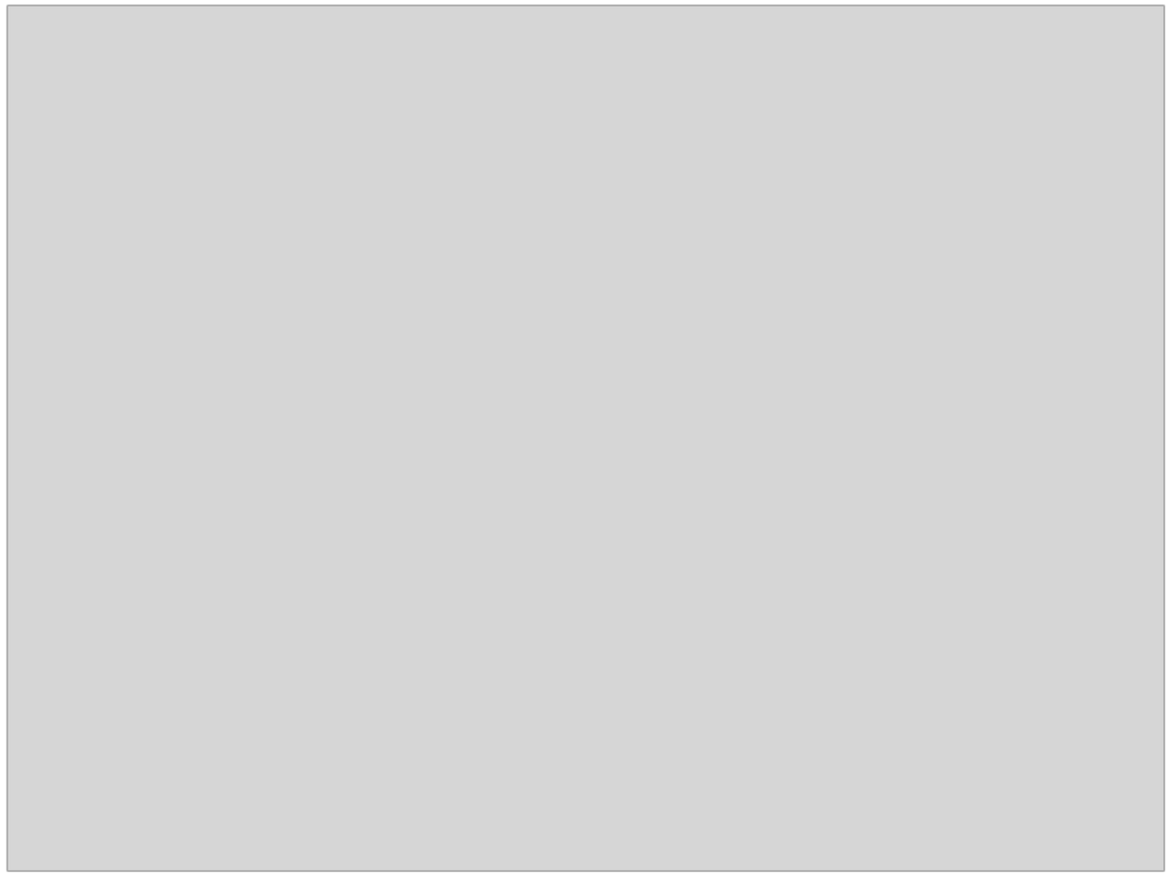
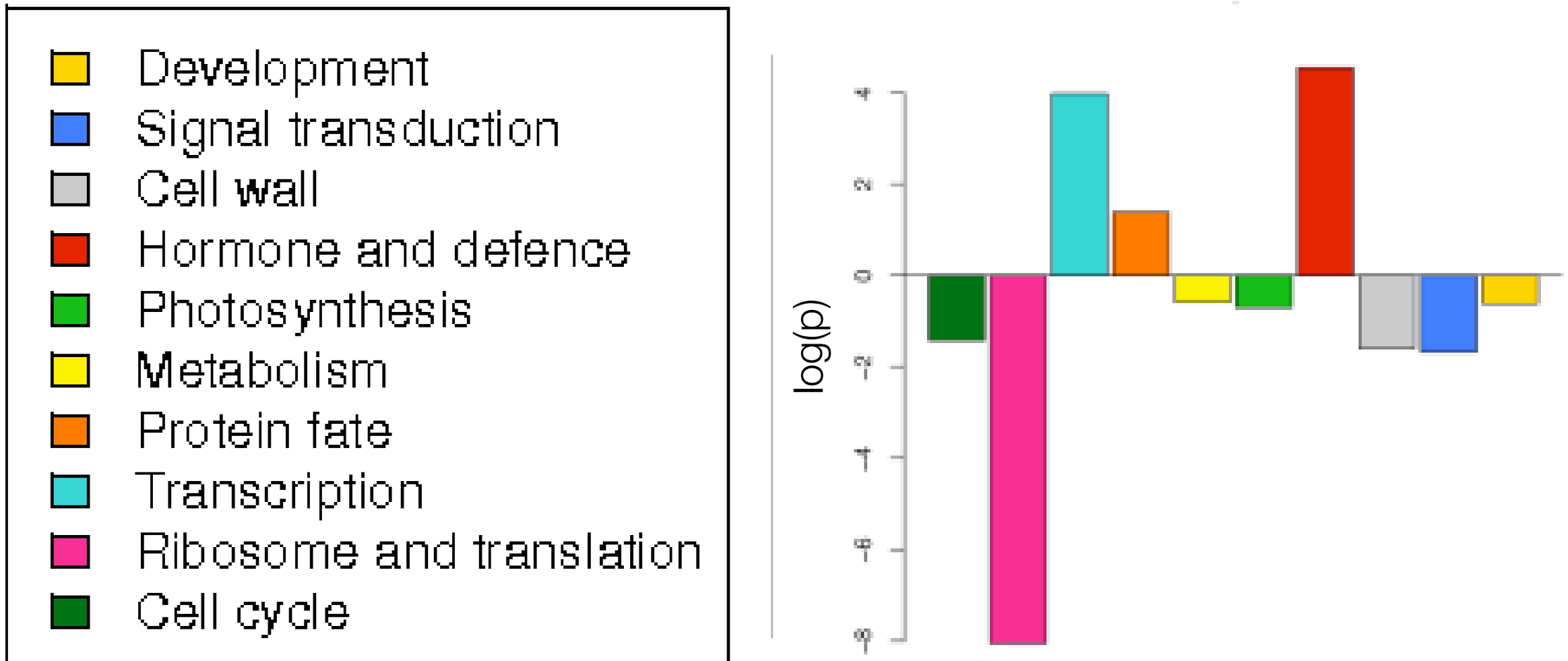
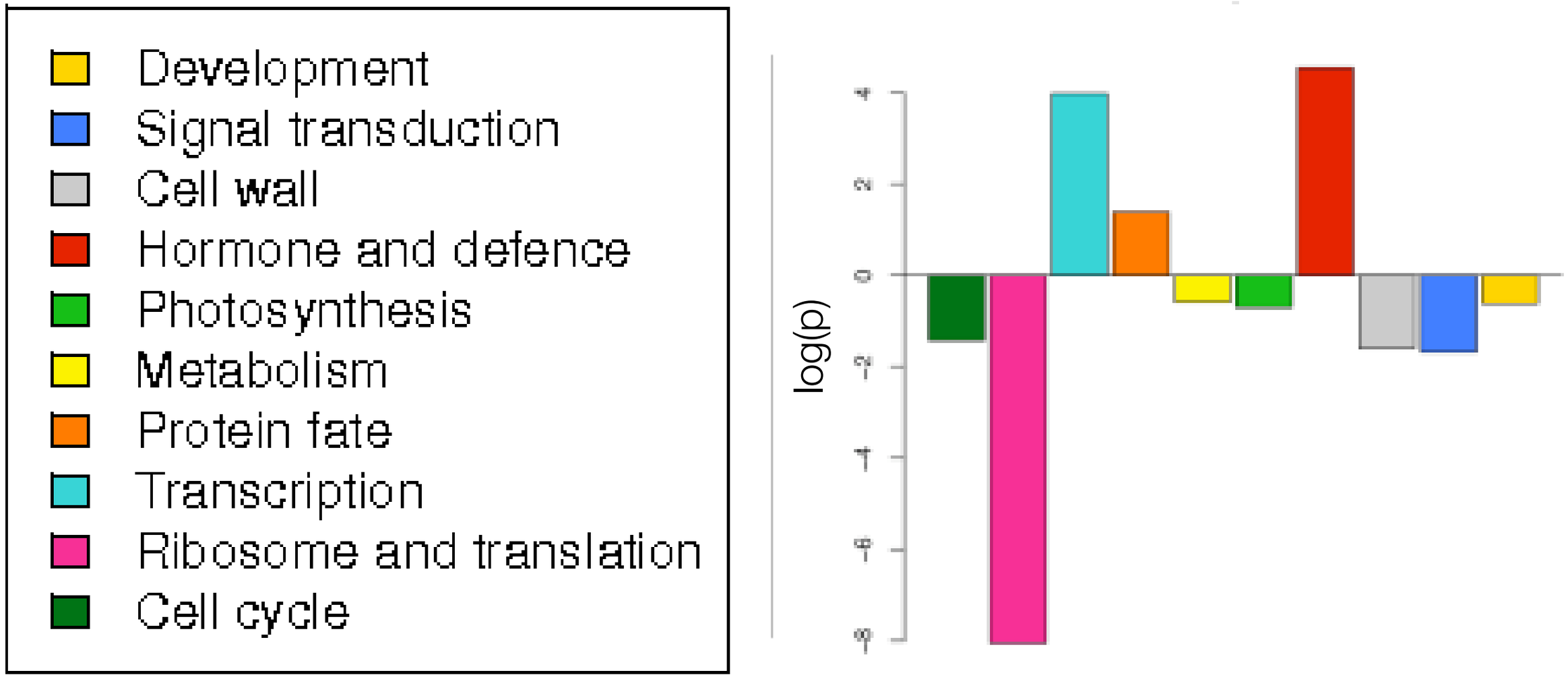




Example


Development
Signal transduction
Cell wall
Hormone and defence
Photosynthesis
Metabolism
Protein fate
Transcription
Ribosome and translation
Cell cycle
log(p)
4
2
0
-2
-4
-6

Example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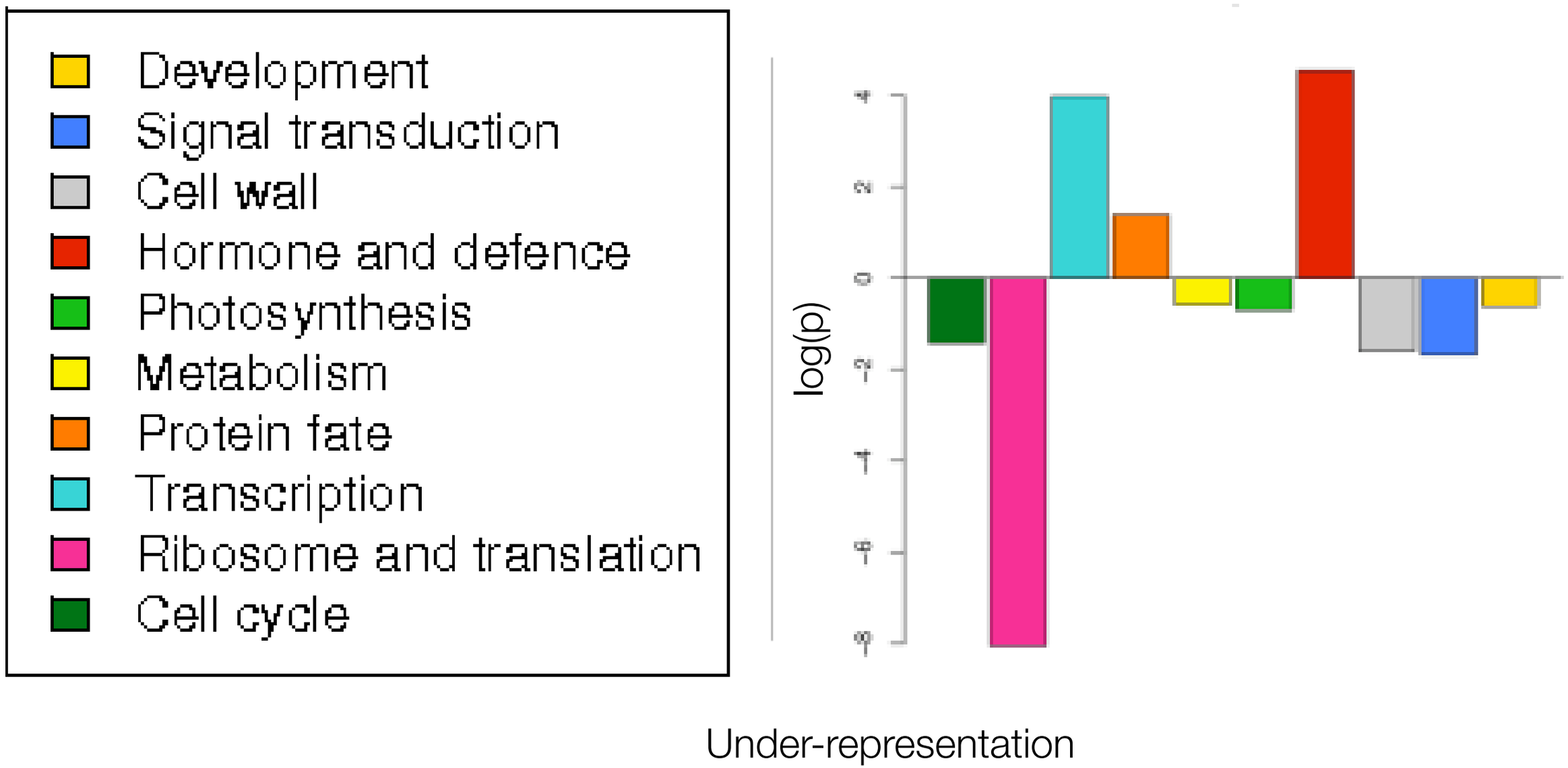
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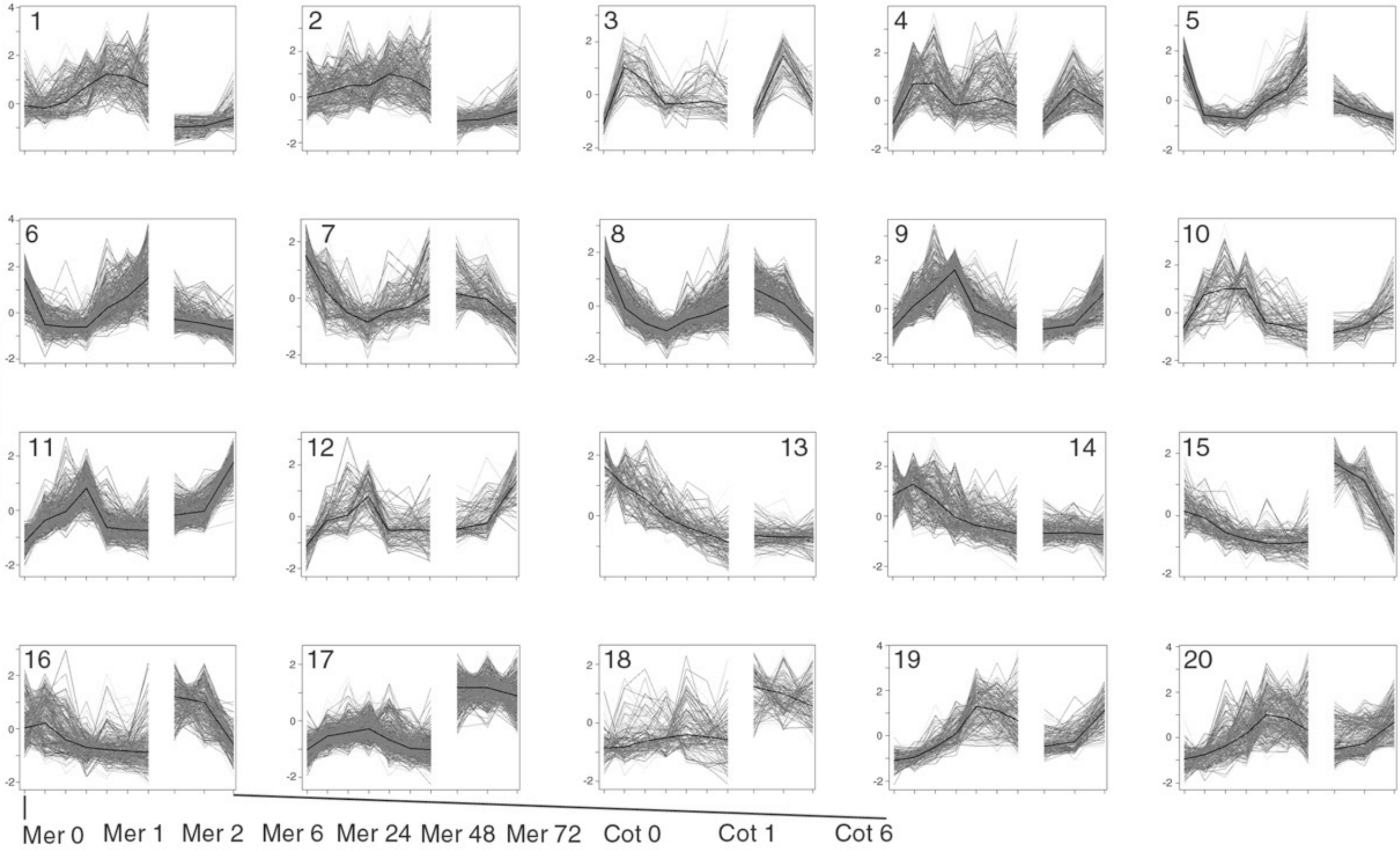
Under-representation

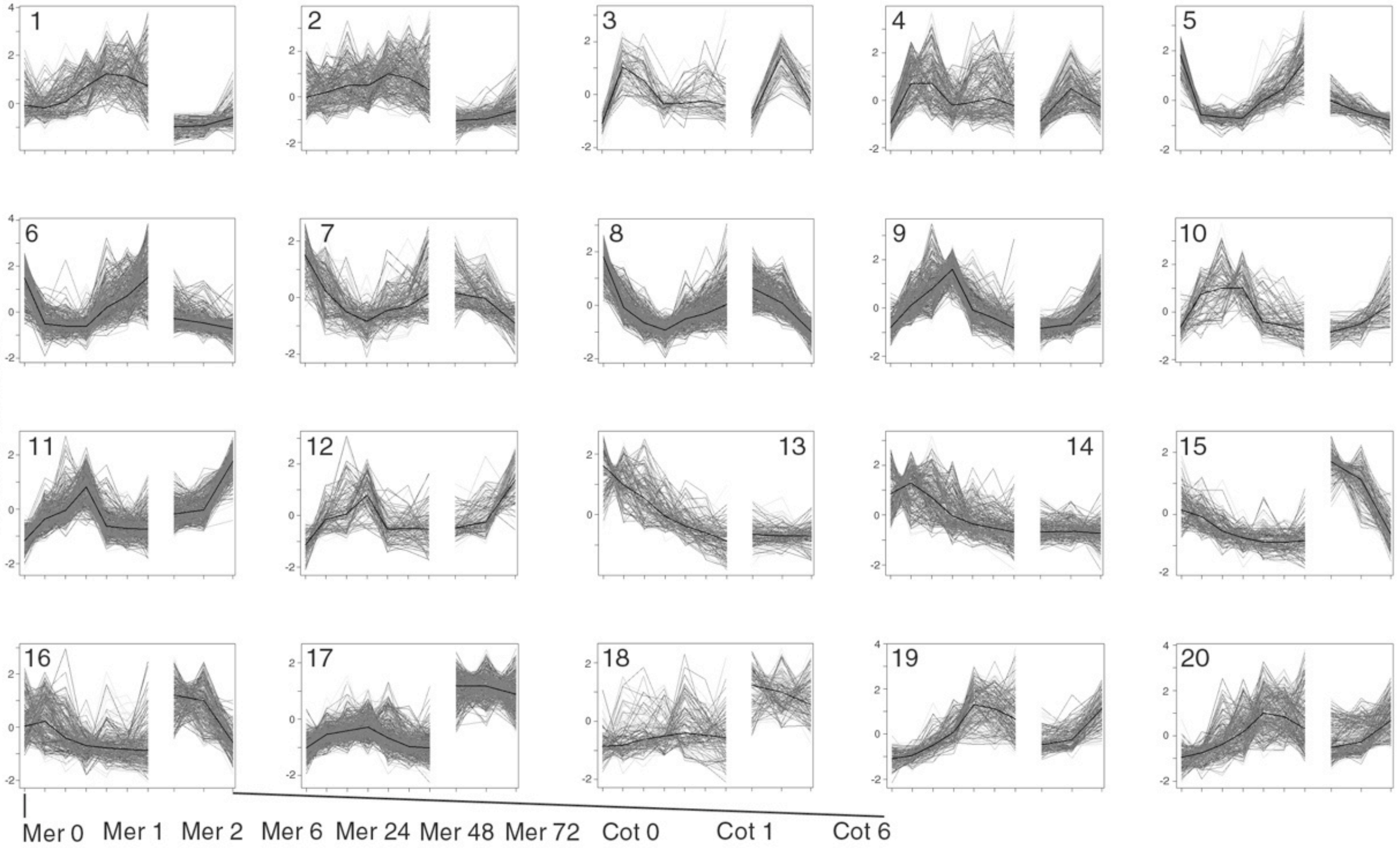
Example

Clustering :- Understanding the time behaviour

- Important lesson :- cluster early, cluster often.
- While genes have been selected using data at 0,1,6 hours, all data is used for clustering.
- Results quoted use K-Means (20 clusters) but checked with varying cluster size and with Hierarchical Clustering (very different clustering algorithm) to check if clusters are consistent.
- For each cluster, examine functional classes and explore any possible over-representations.



K-means clusters (reordered)

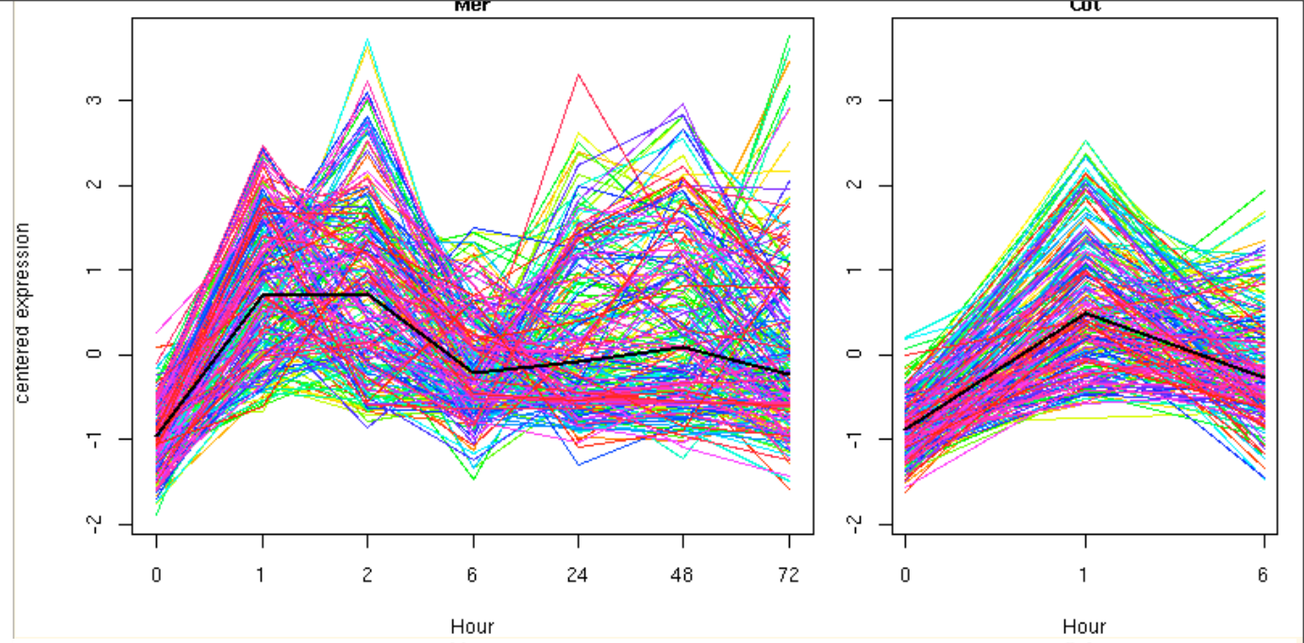


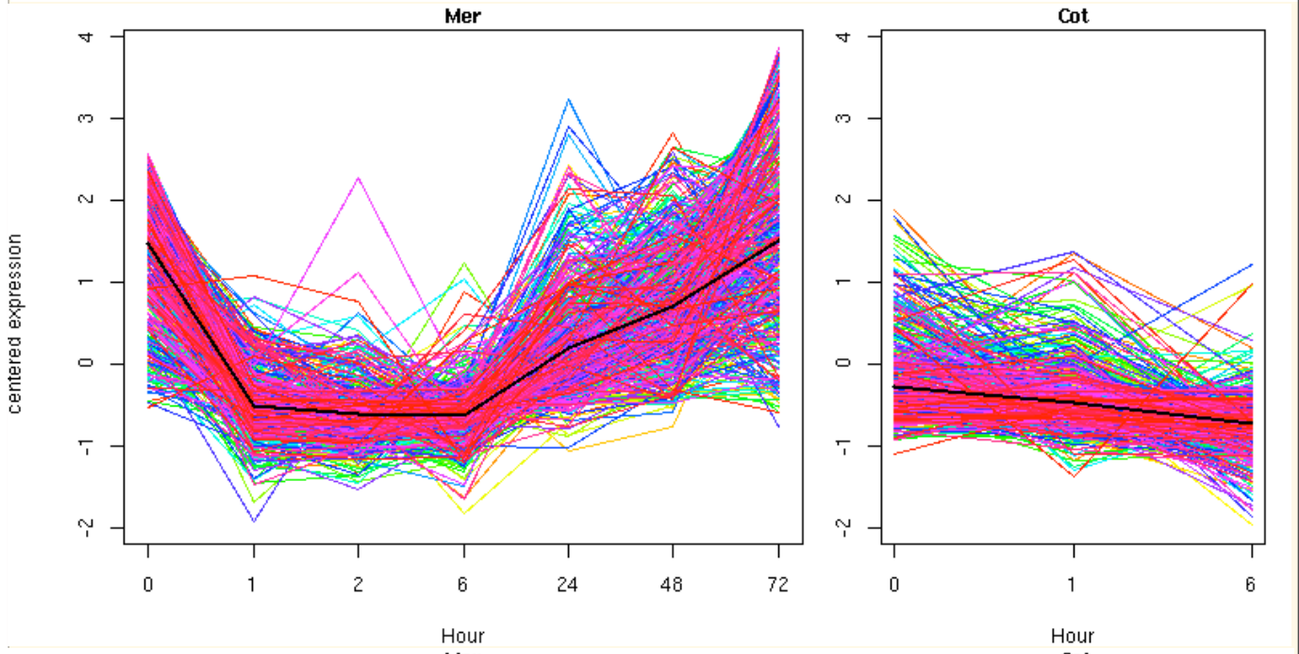
Yes, but what does it mean ?

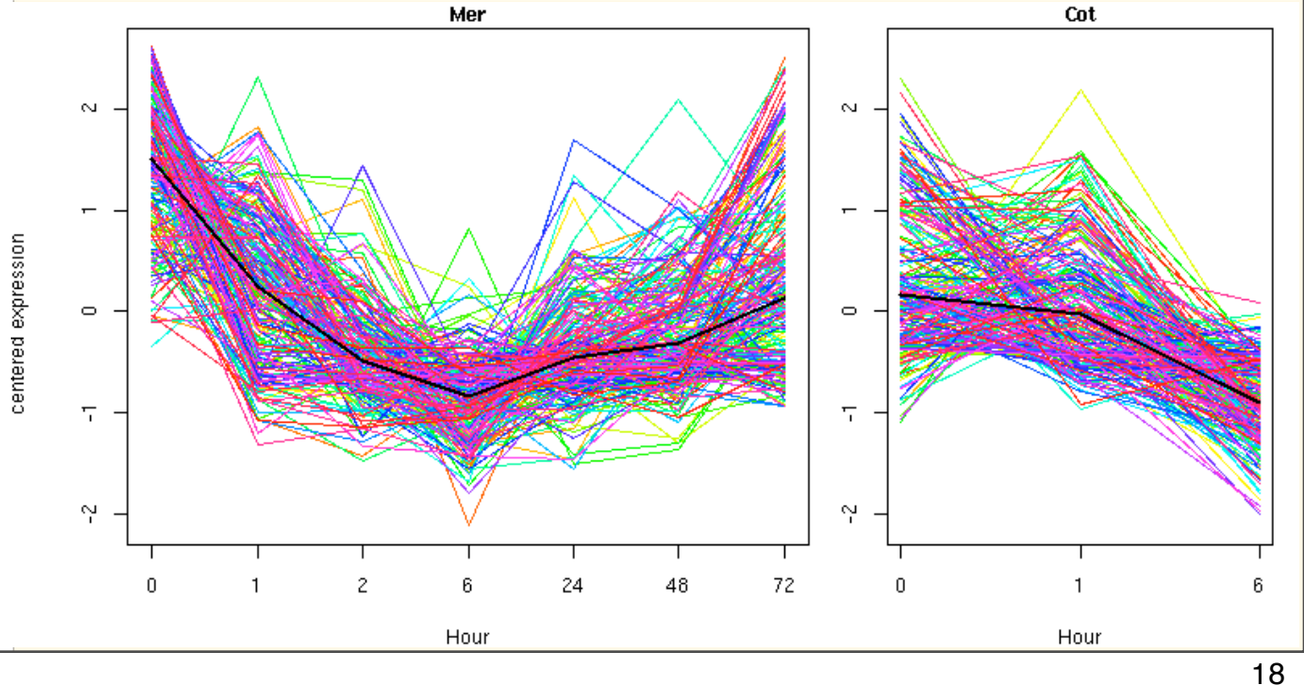
K-means clusters (reordered)

First Phase

- Transcription Factors
- Ubiquitination
- Kinases
- More down-regulation than up.







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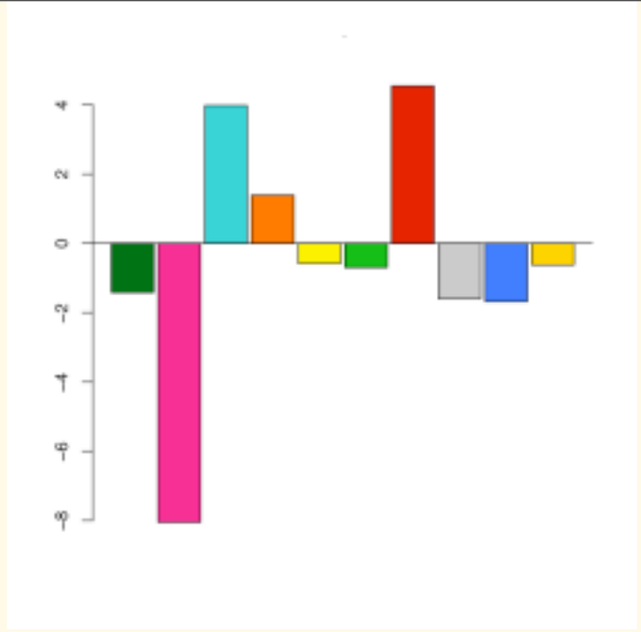

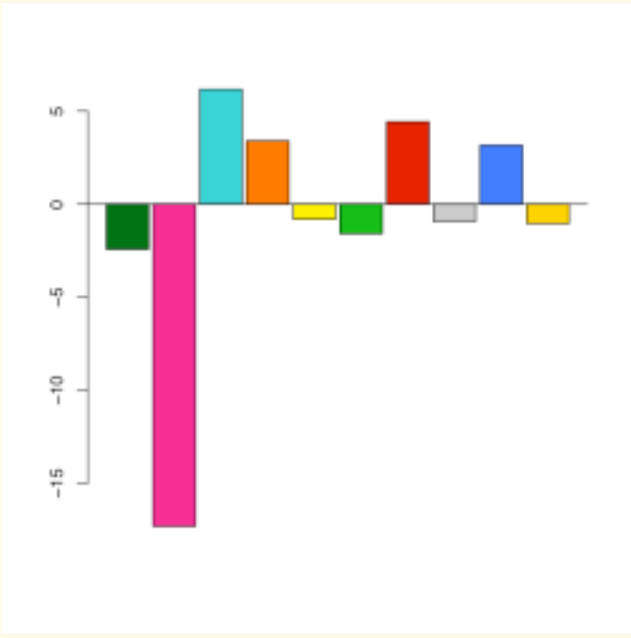

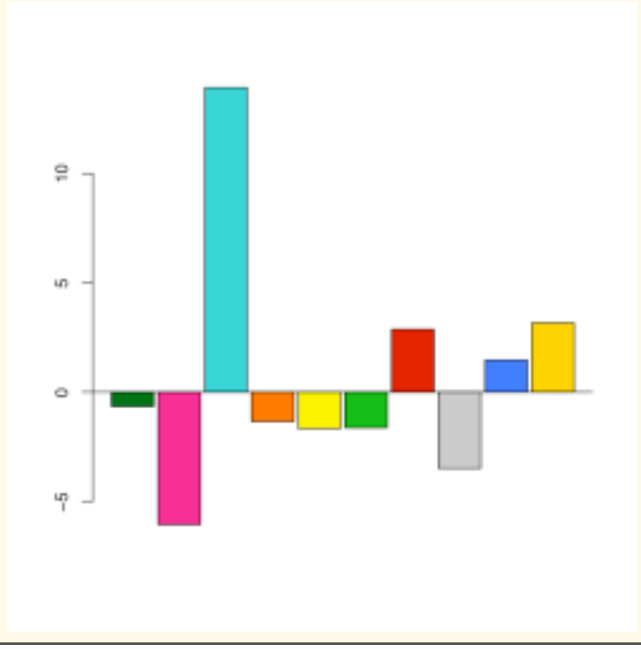

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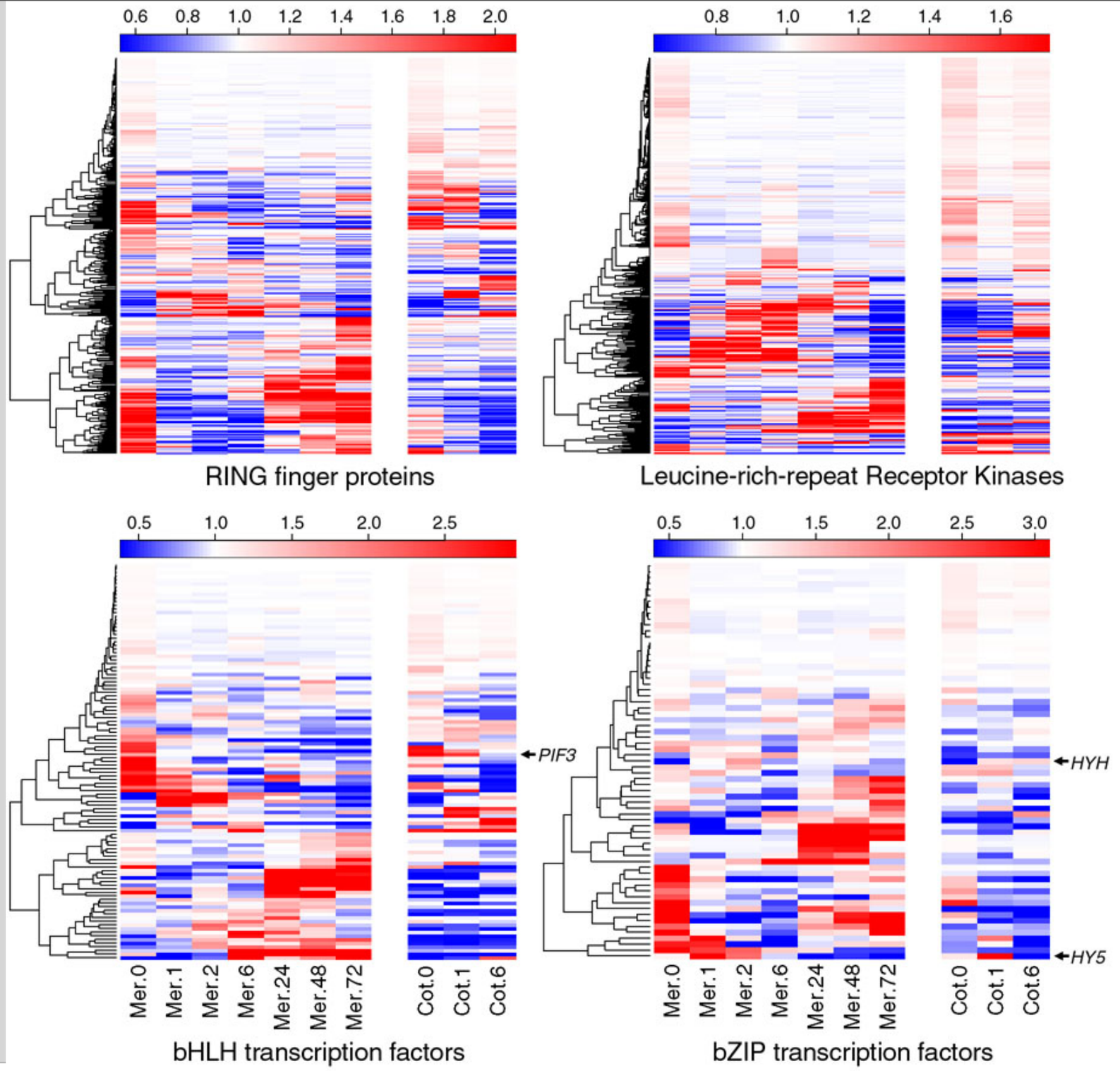
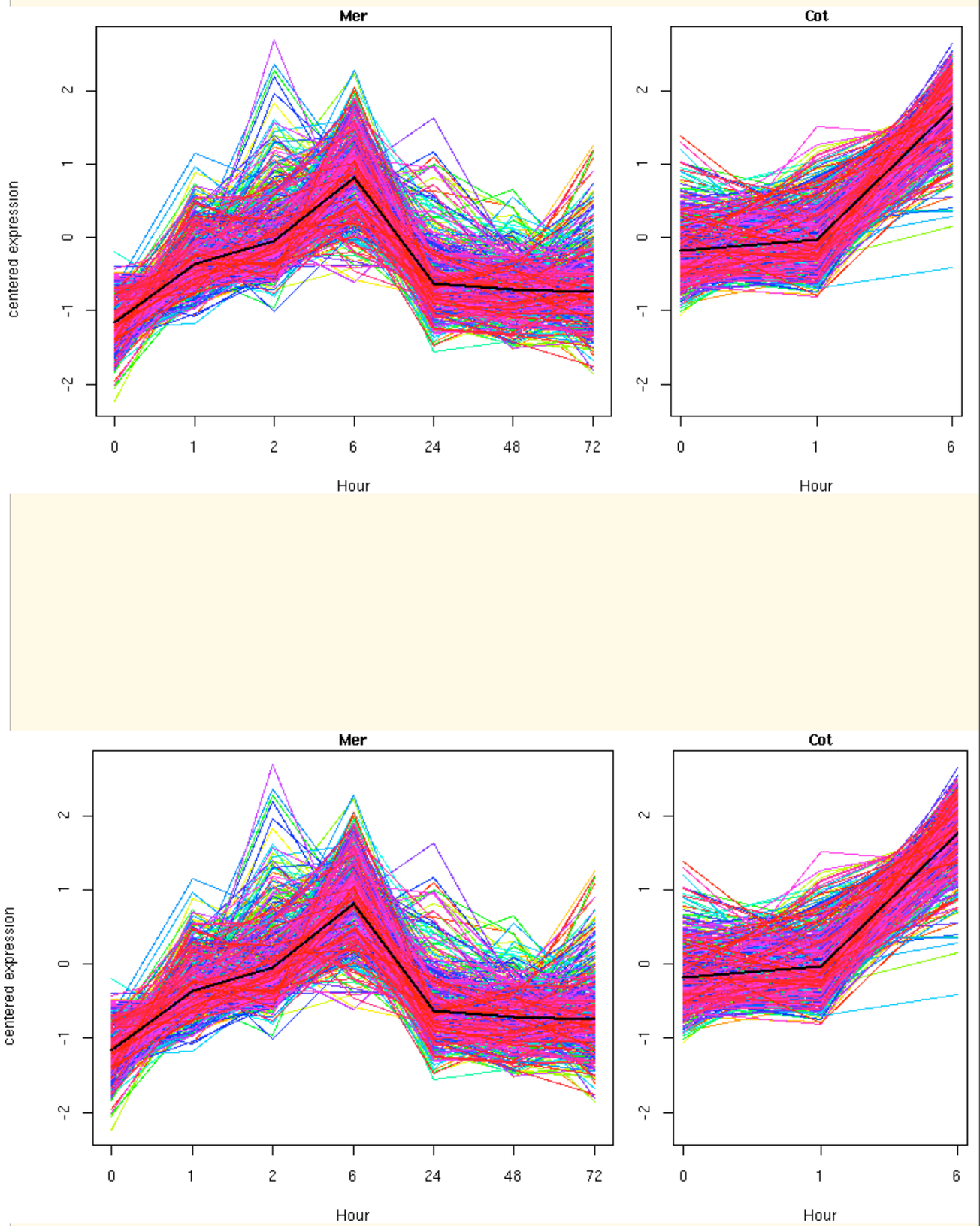


Regulator (?) classes

Including non-selected genes

Second Phase

- Ribosomal activity
- cell cycle
- hormone-related activity



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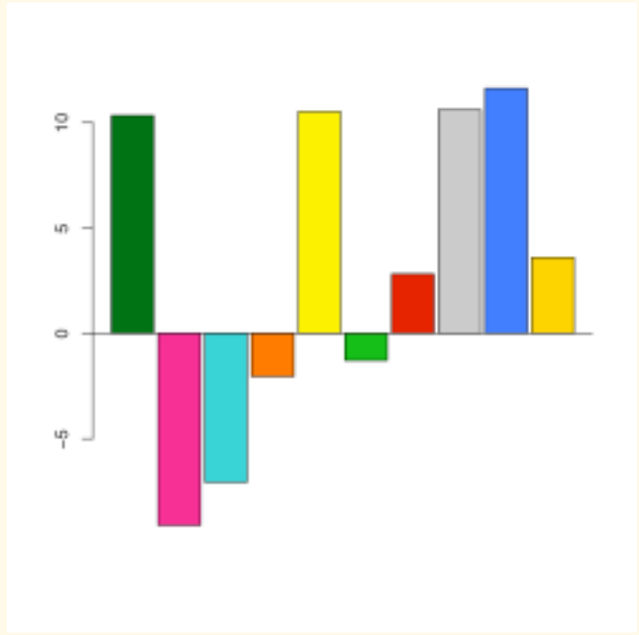
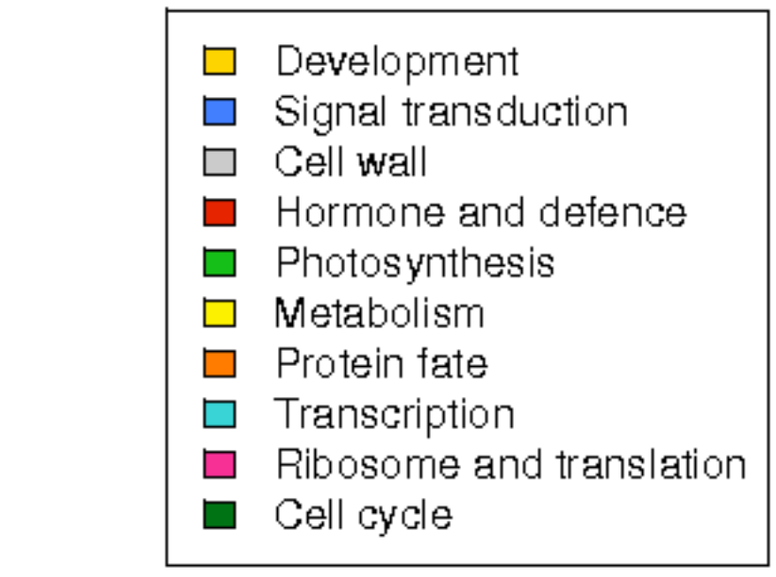
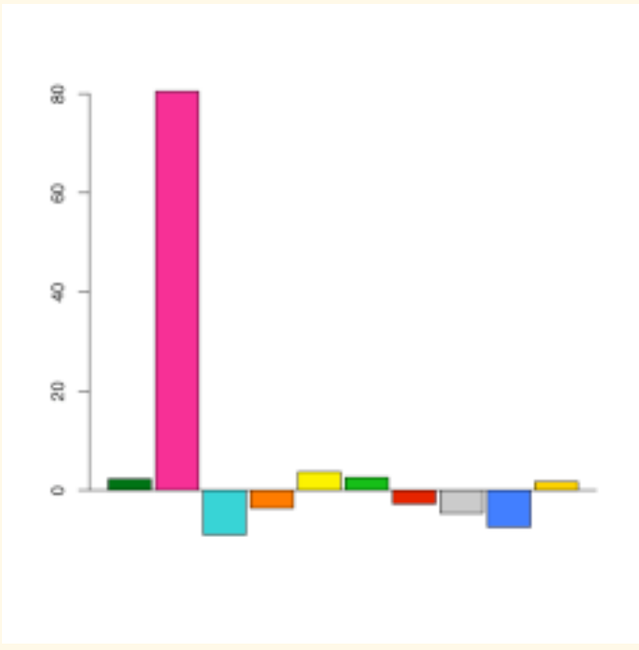
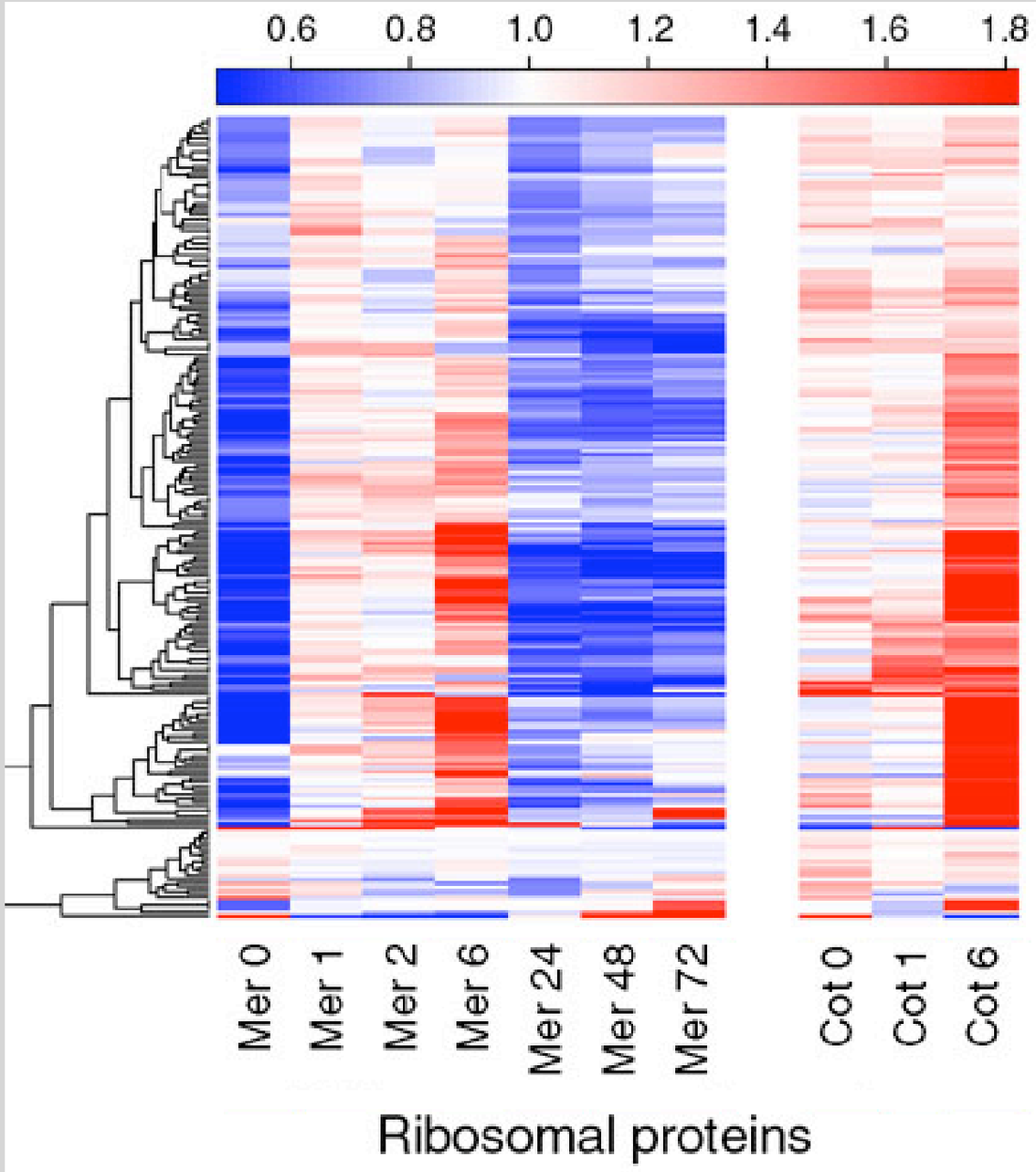
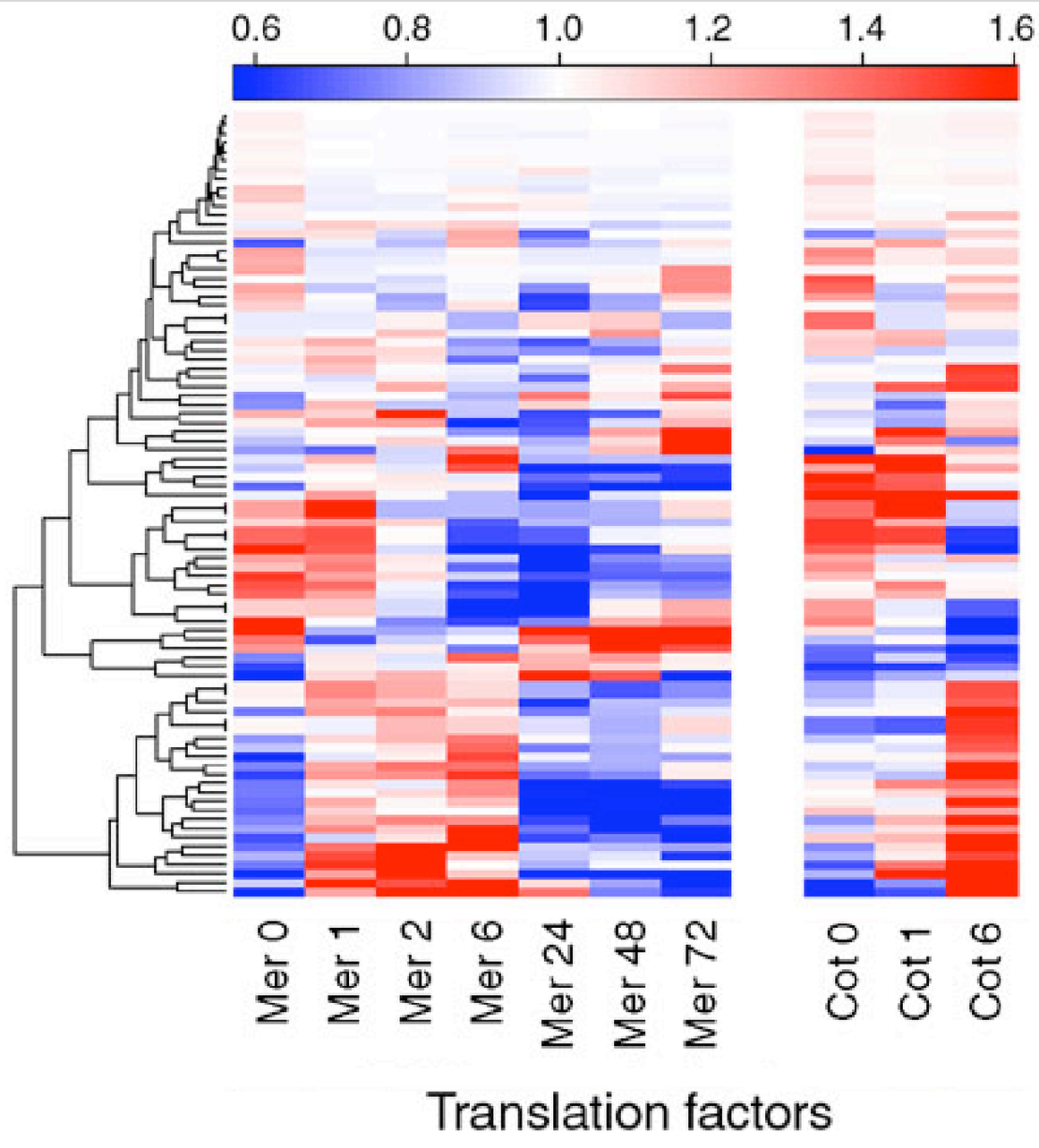






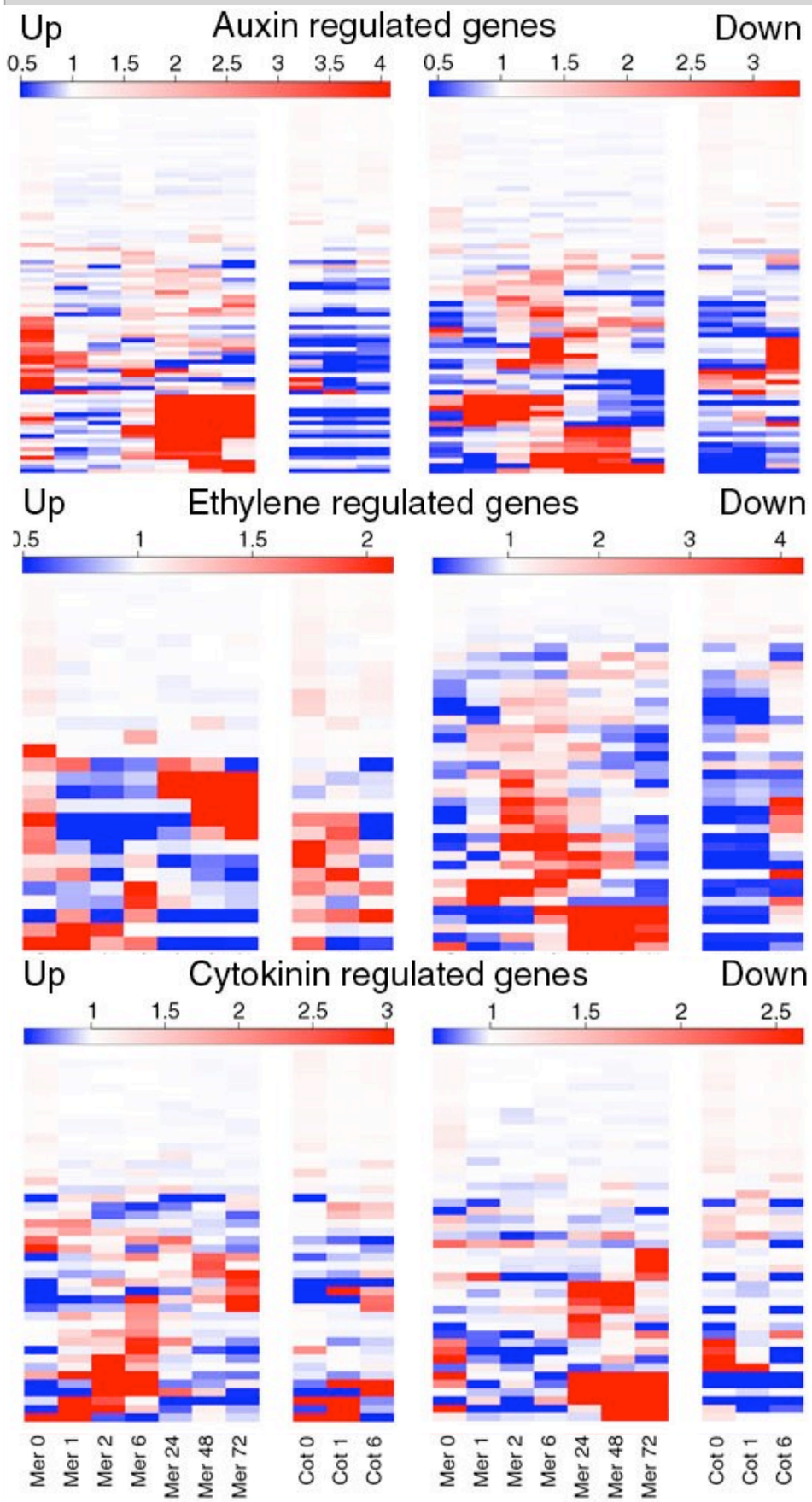




Ribosome working overtime

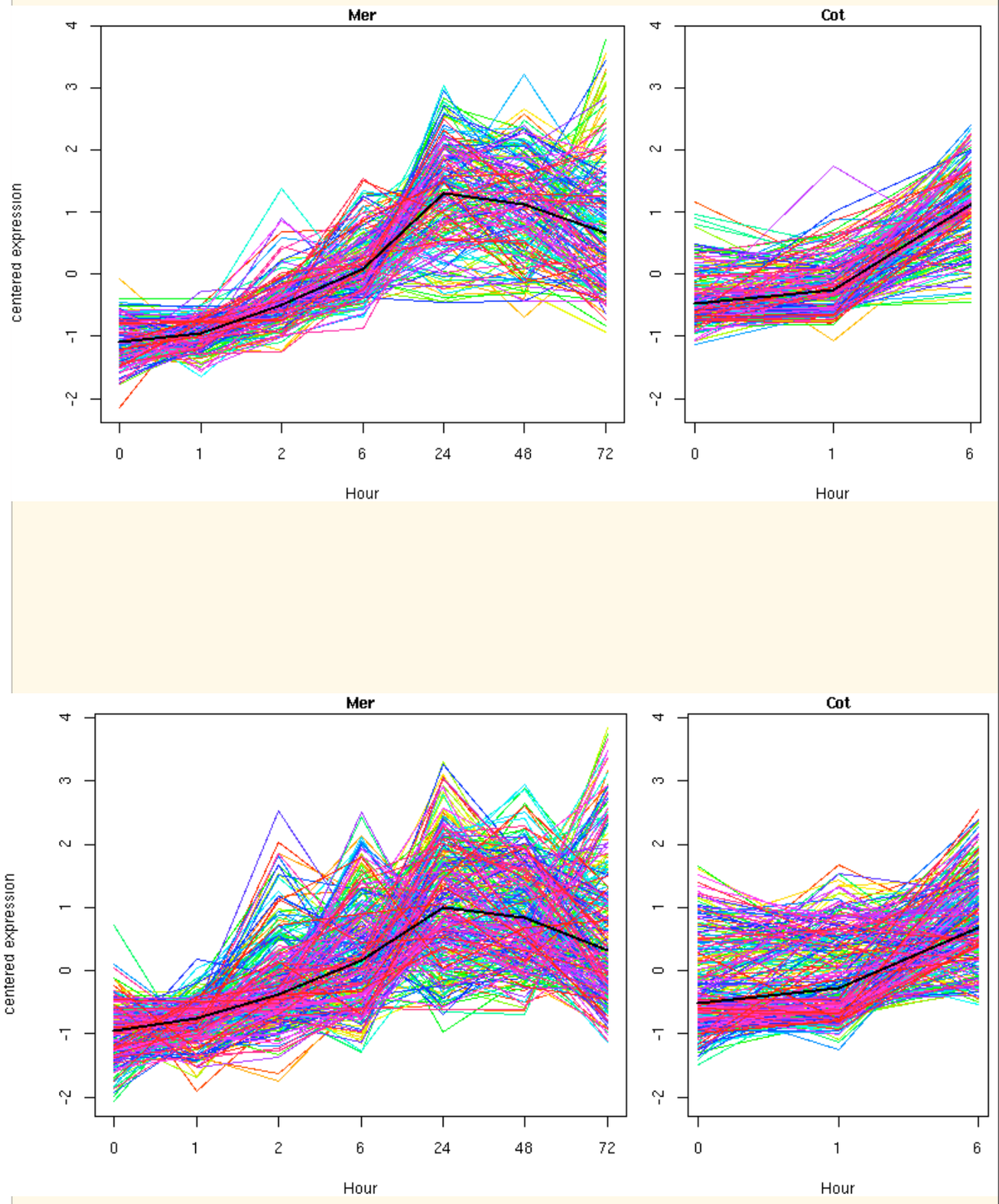
Hormones

- Auxin, Ethylene promote growth (elongation).
- Cytokinin repress elongation.
- Cannot track hormone concs.
- Follow activity of genes regulated by hormones (strictly differentially expressed).
- Auxin, Ethylene “repressed”.
- Cytokinin “promoted”.



Third Phase

- Photosynthesis
- Cell wall loosening



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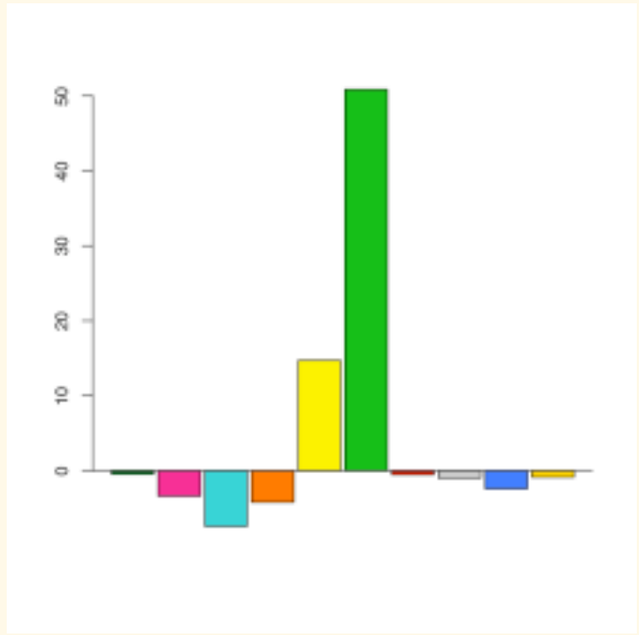
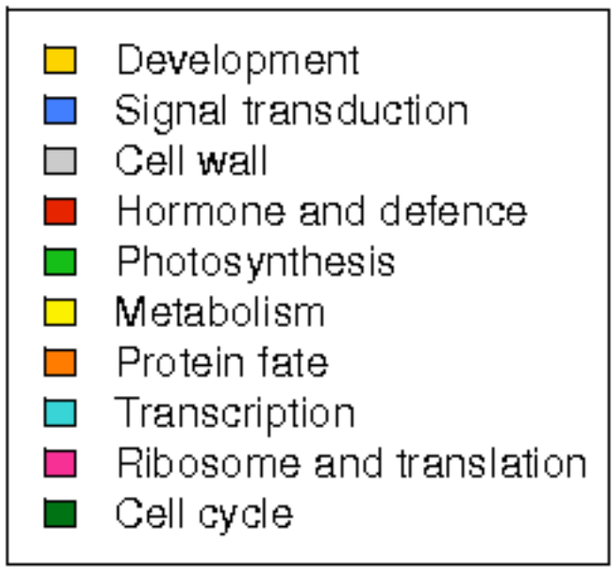
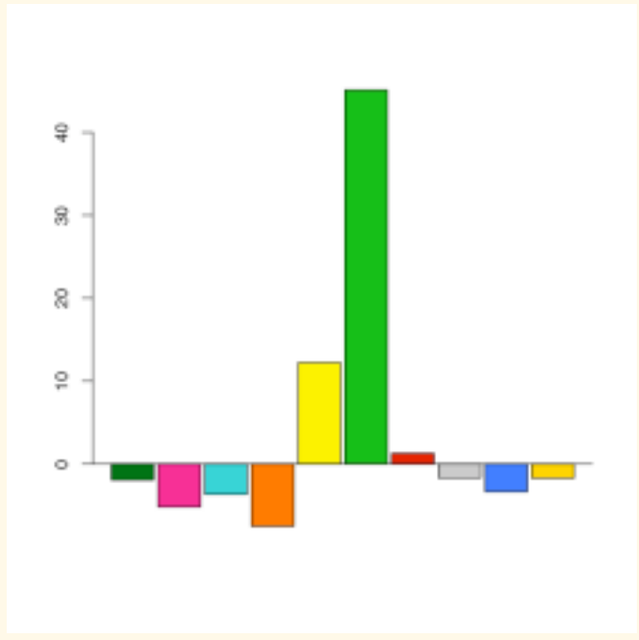
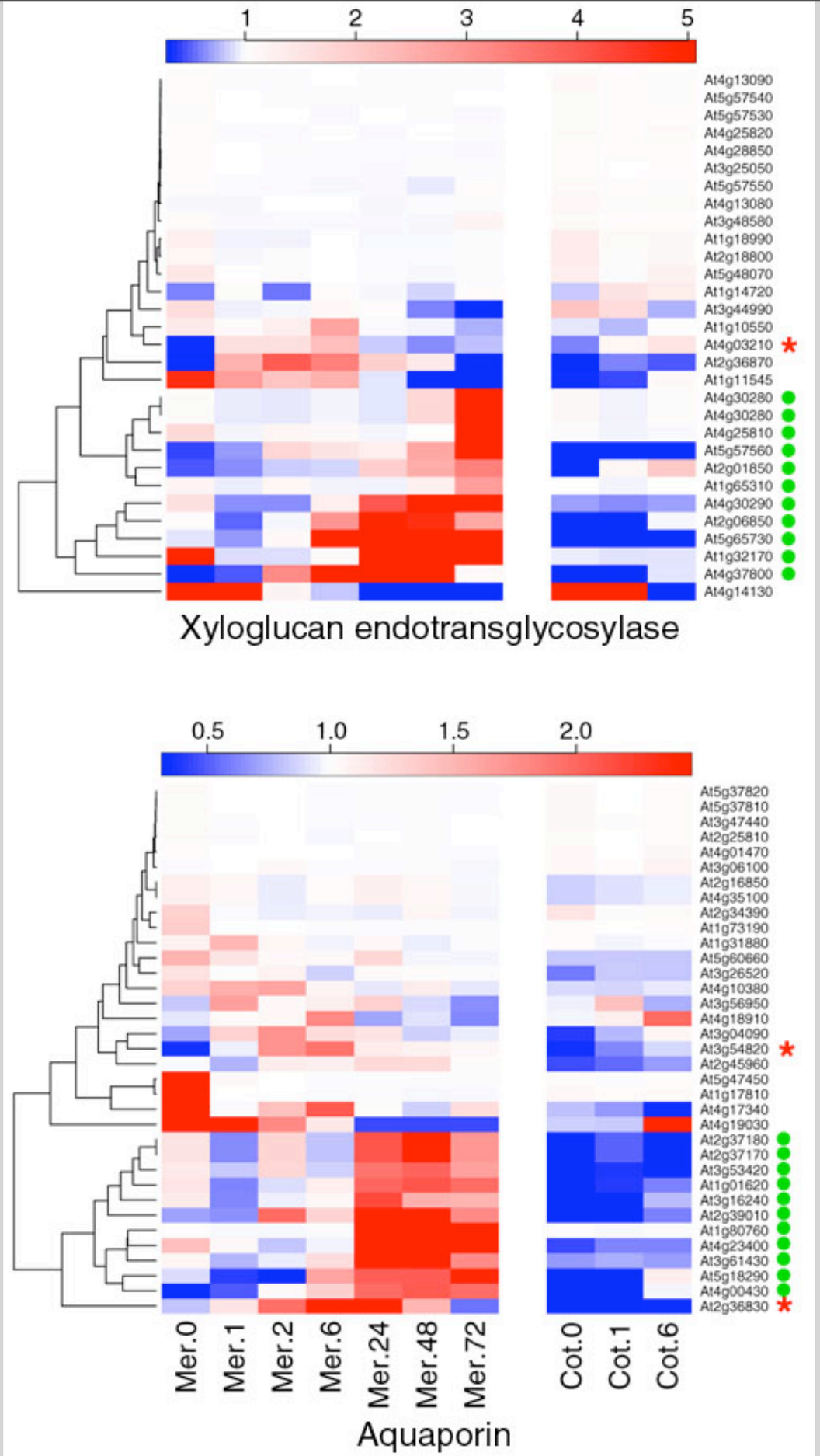






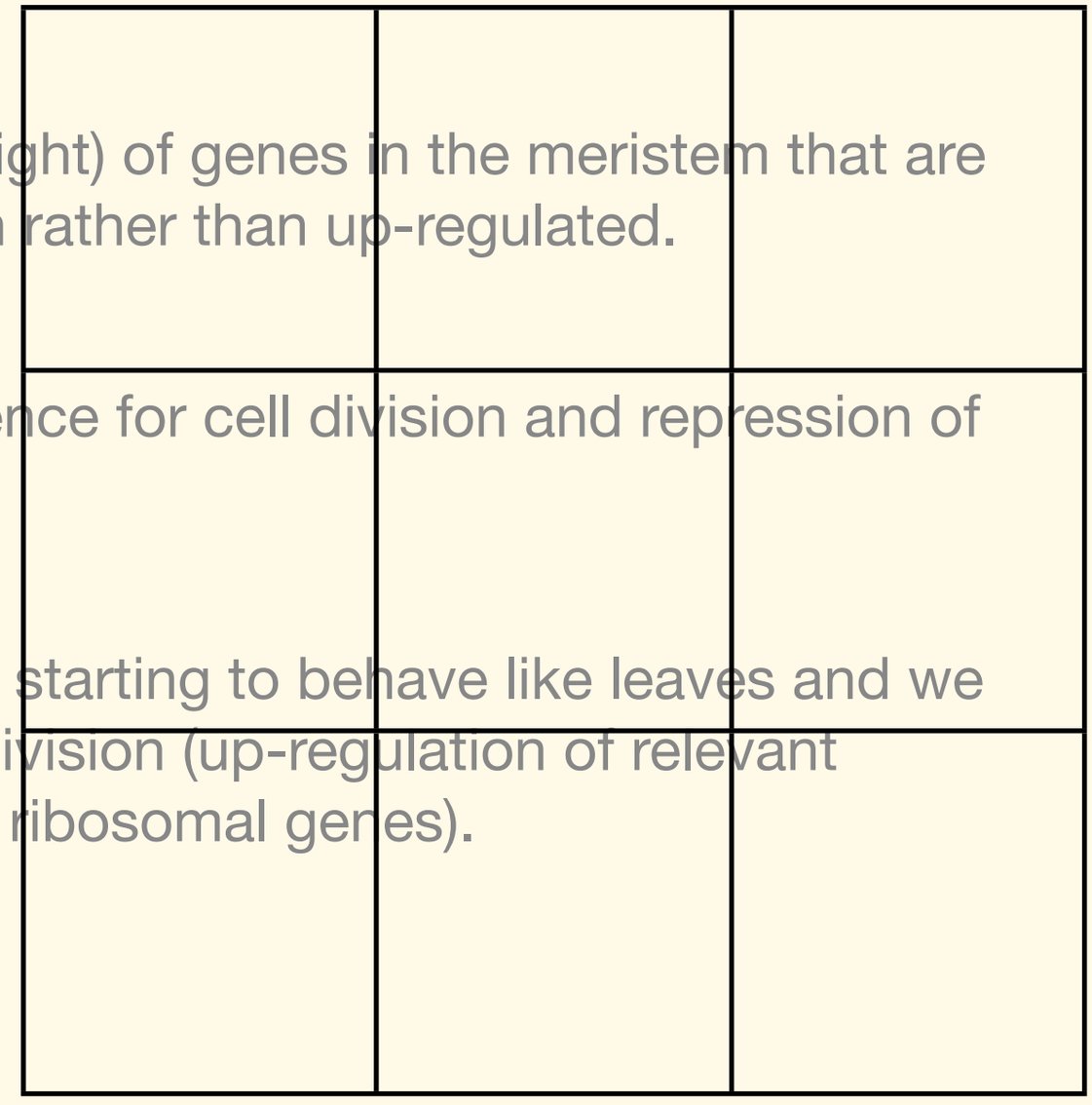
Cell Wall

- Plant cells have rigid cell walls.
- Expansion implies that cell walls must become less rigid.
- Complicated process between cell wall modification and internal turgor pressure.
- Nonetheless, see late expression in genes controlling this behaviour.



Summary of what's been seen

- After constructing a set of genes which are strictly differentially expressed we find:
- An early burst (0-1 hours after exposure to light) of genes in the meristem that are regulatory in nature and are in general down rather than up-regulated.
- Around 6 hours after exposure to light evidence for cell division and repression of growth.
- At later times, parts of meristem are already starting to behave like leaves and we see growth through expansion rather than division (up-regulation of relevant hormone-related genes, down-regulation of ribosomal genes).



Where to go from here

- So far we've put together a picture of genes up and down regulated and looking at their classification fits in with a picture of particular types of growth.

- Useful at middle and late times (6 hours and beyond)

- We see sets of transcription factors, MAP kinases and Ubiquitination-related genes which are over-represented.

- Can we identify targets of these regulators ?

- Start off with co-regulated sets and see if we have common upstream promoter elements.

- How conserved are the above mechanisms elsewhere ?

Acknowledgements

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- Enrique López-Juez
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- Zoltan Magyar
- Laszlo Bögre

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- Saul Hazeldine

Department of Molecular Genetics,
Ghent University

- Gerrit Beemster

Institute of Biotechnology,
University of Cambridge

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