Incorporating Toxicogenomic Data and Approaches in Risk Assessment

Case study: Phthalate Esters

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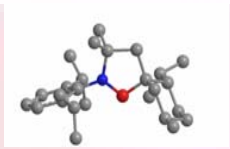
Goals of toxicogenomics

- To understand relationships between environmental exposures and human disease susceptibility;
- To facilitate the application of gene and protein expression technology in toxicological problems;
- To identify useful biomarkers of disease and exposure to toxic substances;
- To improve computational methods for understanding the biological consequences of exposure and responses to exposure; and
- To create public databases of environmental effects of toxic substances in biological systems

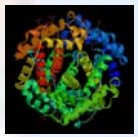
http://www.niehs.nih.gov/nct/

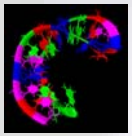
General framework


Test Compound

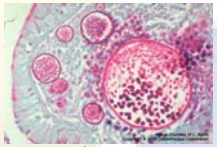

Test Animal


Target Organ


Protein

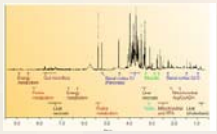

RNA

Histopathology


Cross-Species Extrapolation

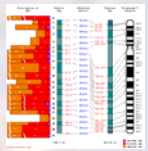
Gene Chip


Metabonomics


DNA


Gene Expression Profiling


SNP-Genotyping


Other Animal Models



Data Integration

Biomarkers for Toxicity

http://www.kitox.re.kr/eng/study/pag_14.jsp

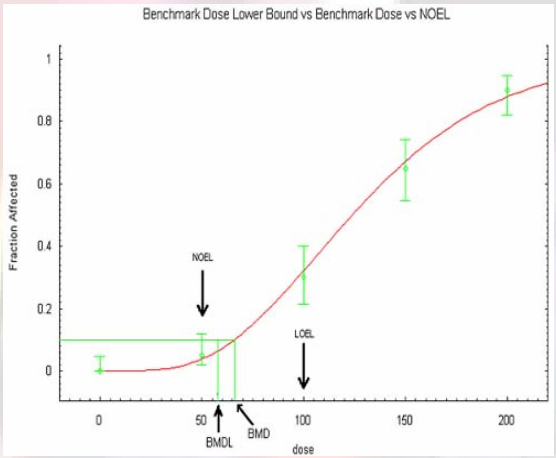
Potential outcomes

•Predictive Toxicology

-Quantitative Risk Assessment

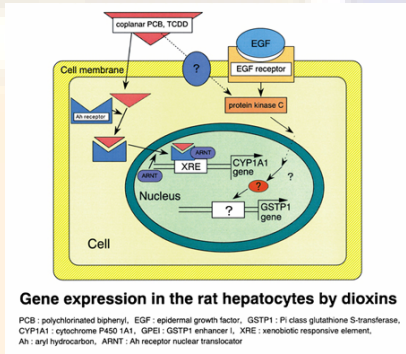
•Dose-Response Assessment

-Extend the dose-response curve



•Mechanistic Understanding

-Putative MoA

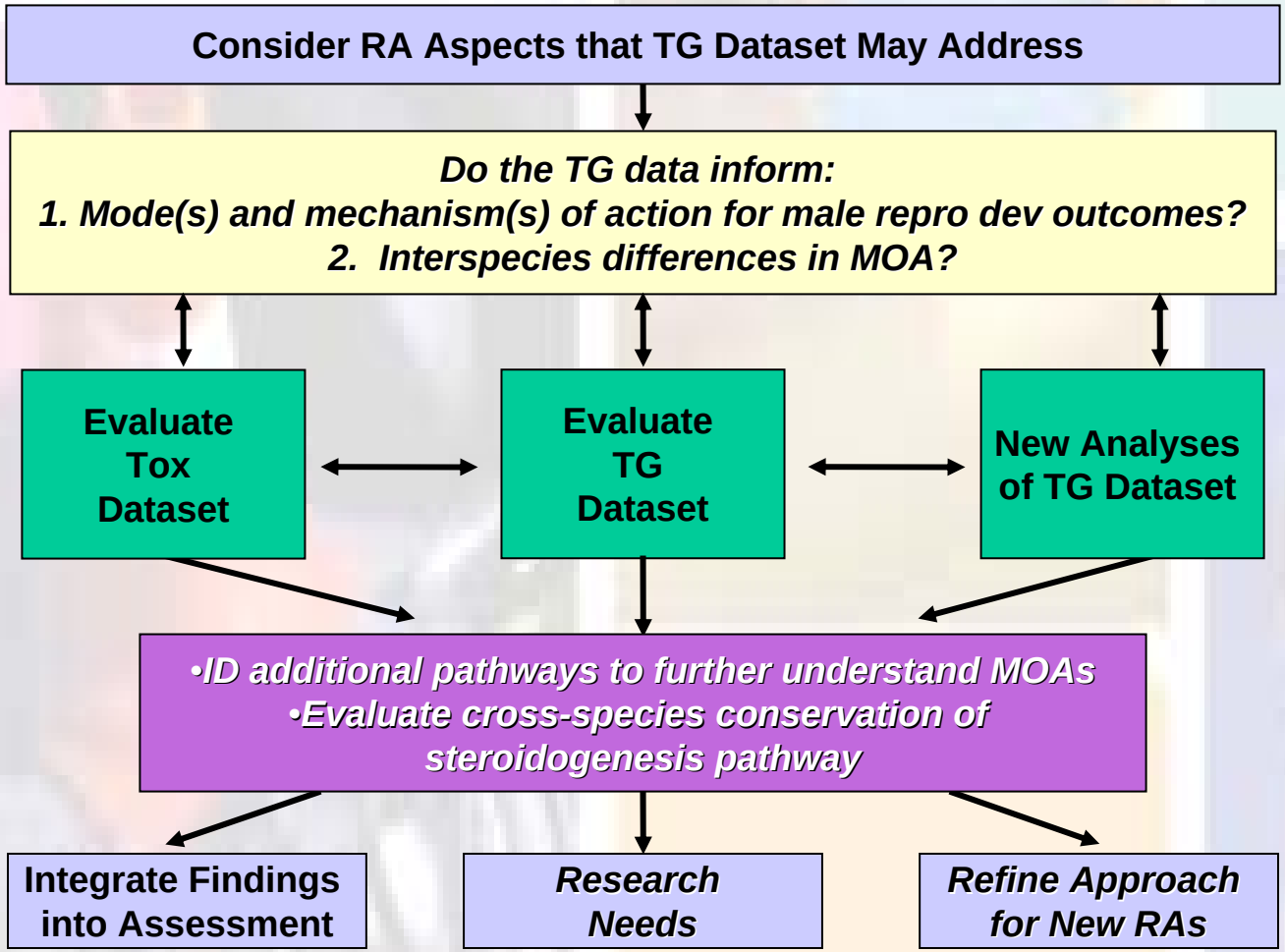


Gene expression in the rat hepatocytes by dioxins

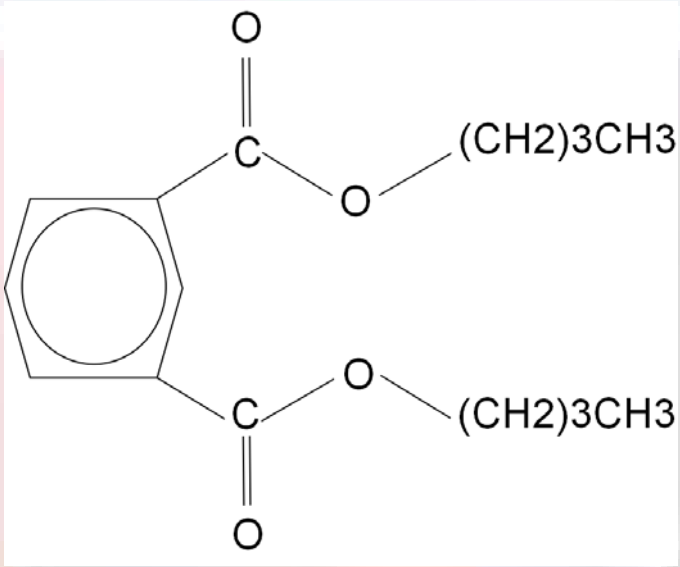
PCB : polychlorinated biphenyl, EGF : epidermal growth factor, GSTP1 : Pi class glutathione S-transferase, CYP1A1 : cytochrome P450 1A1, GPEI : GSTP1 enhancer I, XRE : xenobiotic responsive element, Ah : aryl hydrocarbon, ARNT : Ah receptor nuclear translocator

Toxicogenomics in risk assesment

Di-n-butyl phthalate (DBP) case study



DBP exposure implications



- DBP (Di-butyl-pthalate) is a synthetic, odorless and colorless chemical additive used as a softener of hard plastics
- Potential of human exposure exists because of widespread use and occurrence in environment, as well as high production volumes[1]
- Substantial literature addresses reproductive and developmental effects[2,3,4]

1. Kavlock, R. et al., NTP CERHR Mon, 2003(4); 2. Ema, M. et al., Toxicology, 1994. 86(3);
3. Ema, M. et al., Toxicol Lett, 1998. 98(1-2); 4. Parks, L.G. et al.,Toxicol Sci, 2000. 58(2)

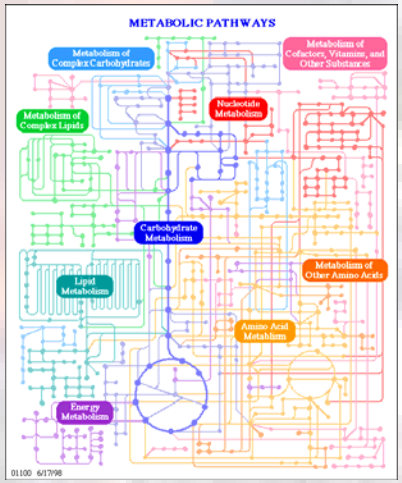
DBP mode of action

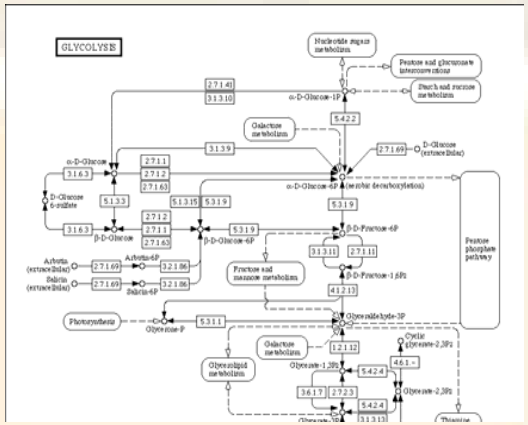
- Interferes with mechanisms involved in reproductive organ development
  - DBP does not bind to the androgen receptor (AR) as flutamide[5]
  - DBP reduces testosterone synthesis by decreased gene expressions in cholesterol transport and steroid biosynthesis pathways[6,7]
  - DBP affects intracellular lipid and cholesterol homeostasis, insulin signaling, transcriptional regulation and oxidative stress[8]

5. Shultz, V.D. et al., Toxicol Sci, 2001. 64(2); 6. Barlow, N.J. et al., Toxicol Sci, 2003. 73(2);
7. Liu, K. et al., Biol Reprod, 2005. 73(1); 8. Thompson, C.J. et al., Biol Reprod, 2005. 73(5)

Main goal of the study

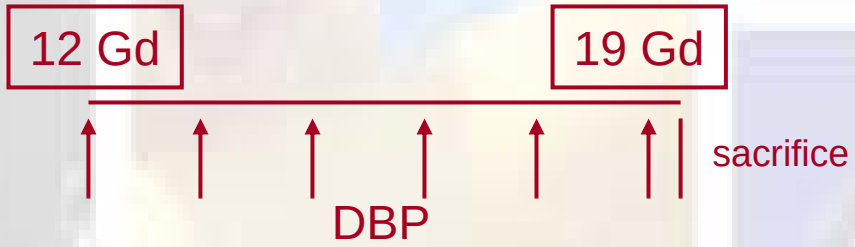
- The main goal is to explore potential MoA, pathways, and regulatory networks using toxicogenomics data and explore whether additional pathways are affected beyond testosterone biosynthesis and insl3 signaling.
- The methodology aims towards the determination of differently expressed pathways



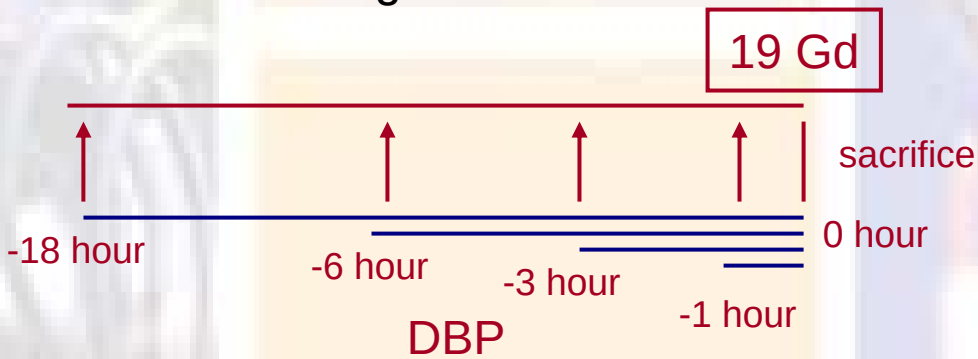


Experimental data

– Rats were treated from gestation day (gd) 12 to gd 19 with corn oil (1ml/kg) and DBP (500mg/kg) per day and sacrificed on 19gd[8]



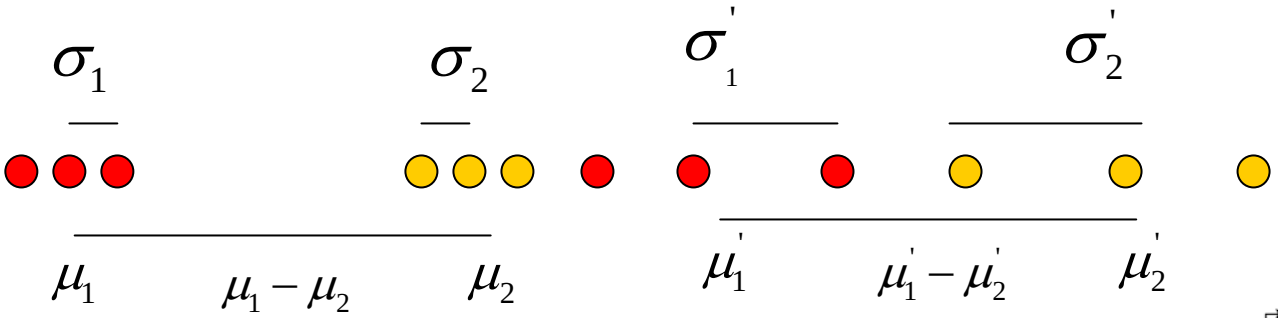
– DBP solutions (500 mg/ml corn oil) were administered to pregnant rats and at the, 1st,3rd, 6th, 18th hour before they were all simultaneously sacrificed at their 19th gd[8]



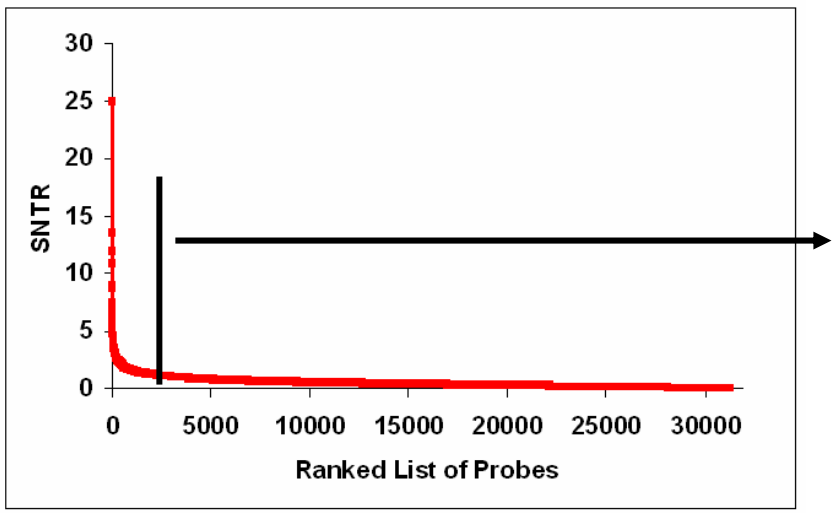
8. Thompson, C.J. et al., Biol Reprod, 2005. 73(5)

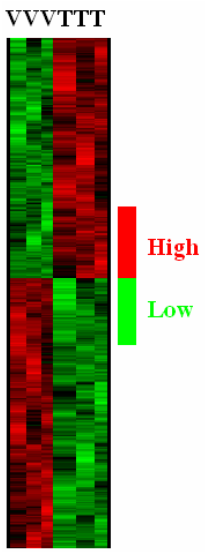
Selection of discriminating genes

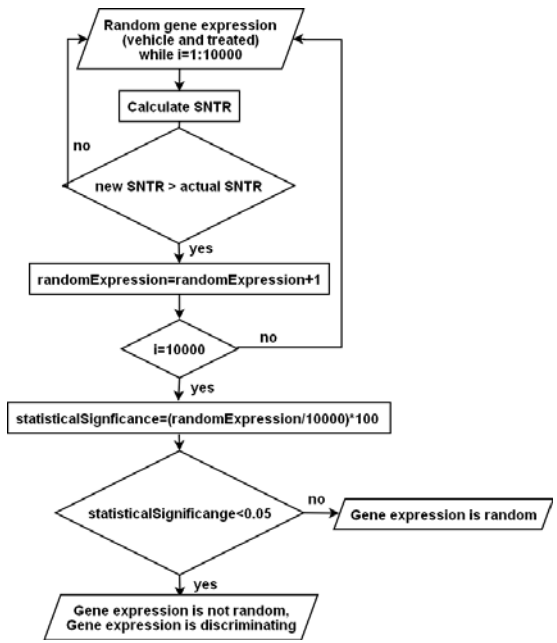
- Selecting Discriminating genes[9]



$$SNTR = \frac{|\mu_1 - \mu_2|}{(\sigma_1 - \sigma_2)}$$







9. Mootha, V.K. et al.,. Nat Genet, 2003. 34(3)

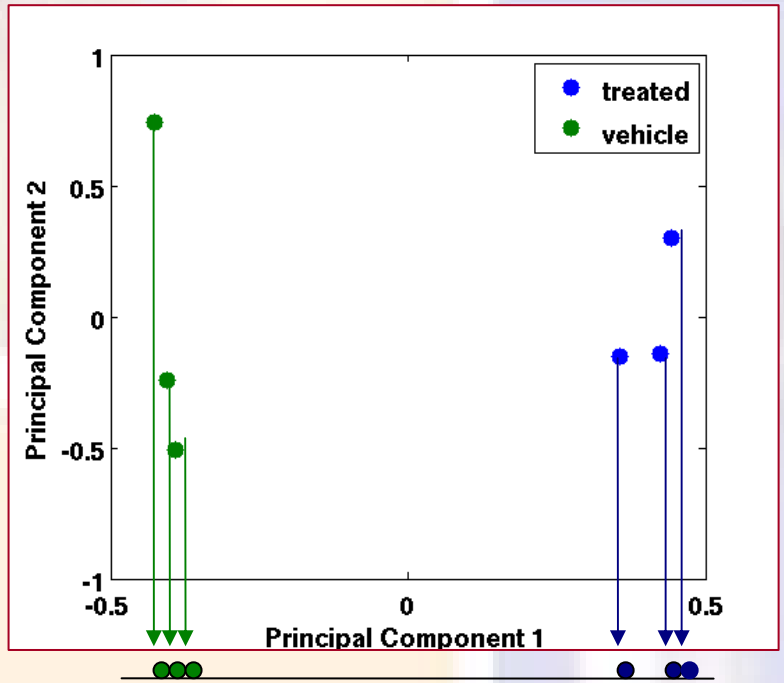
Evaluating pathway activity

- Why Pathway Analysis
  - Consider differentially expressed genes in the context of being part of an active pathway[10]
  - Consider an ensemble of related genes as opposed to individual genes

SVD Analysis

$$X = USV^{I}$$

$$OPA = \frac{|\mu_1 - \mu_2|}{(\sigma_1 - \sigma_2)}$$



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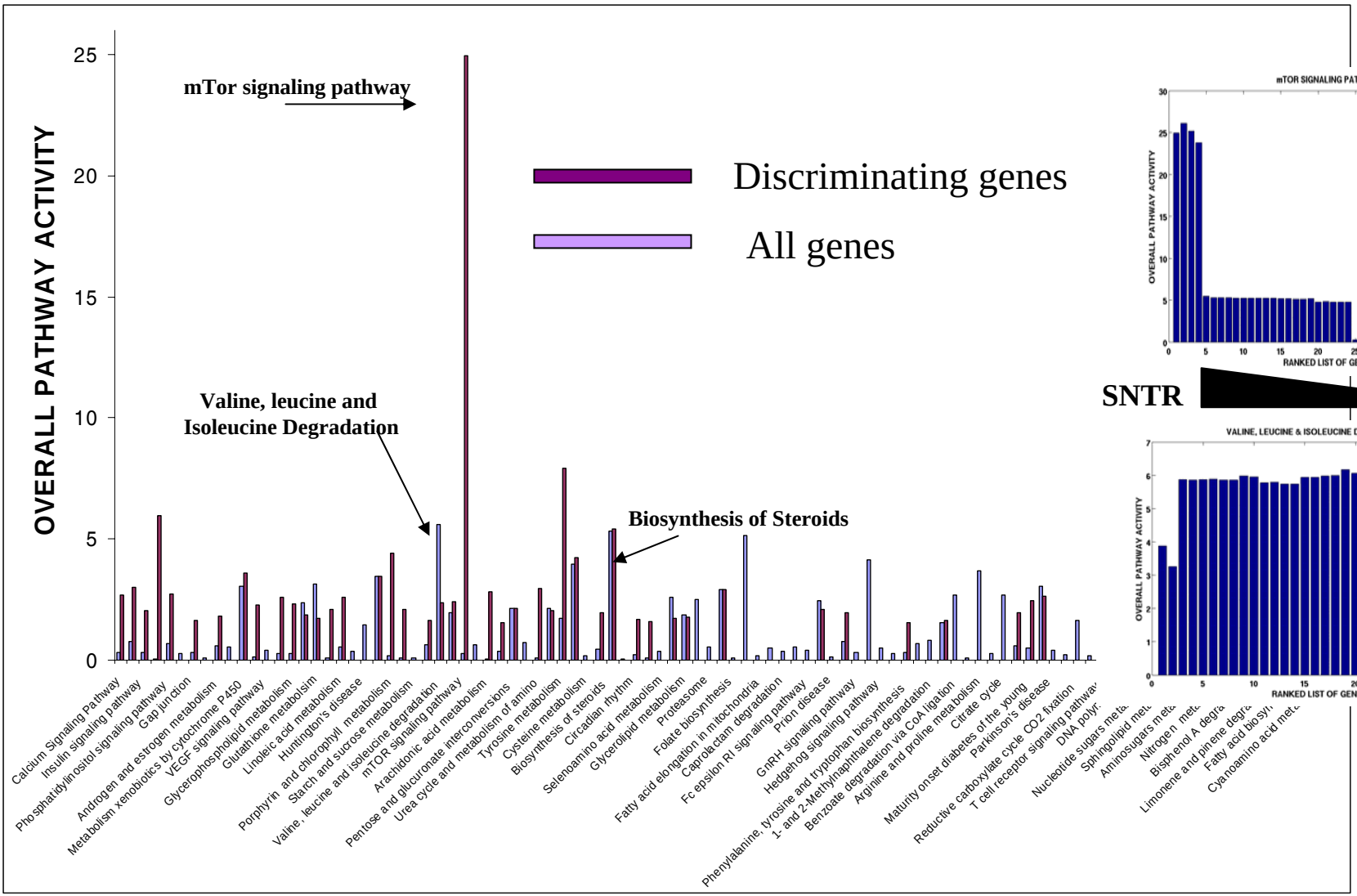
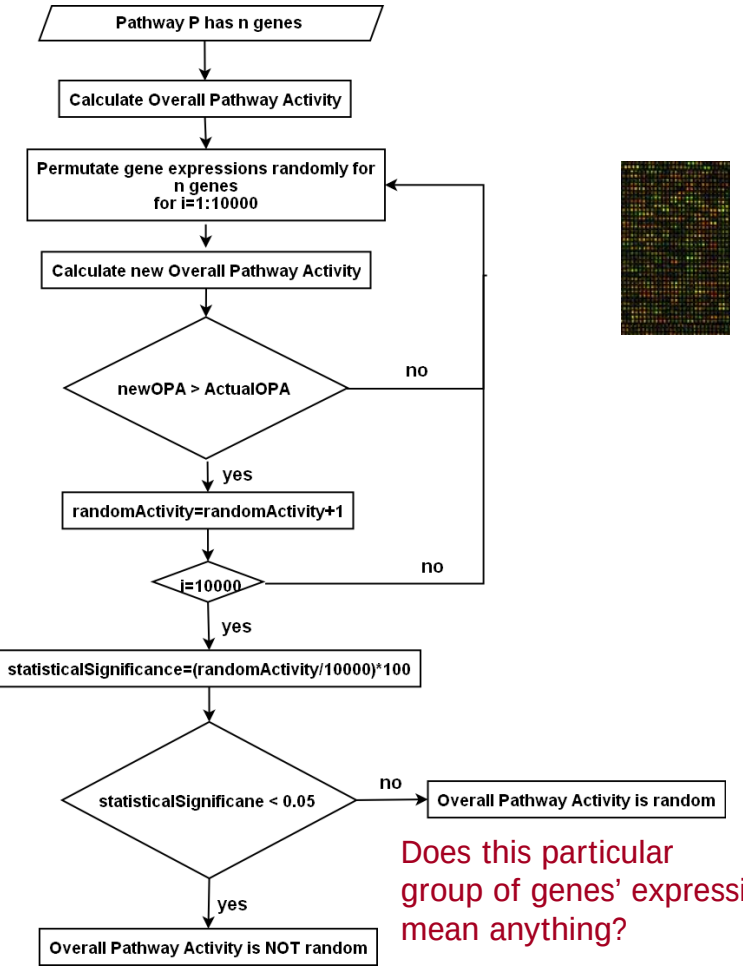
10. Tomfohr, J. et al., BMC Bioinformatics, 2005. 6

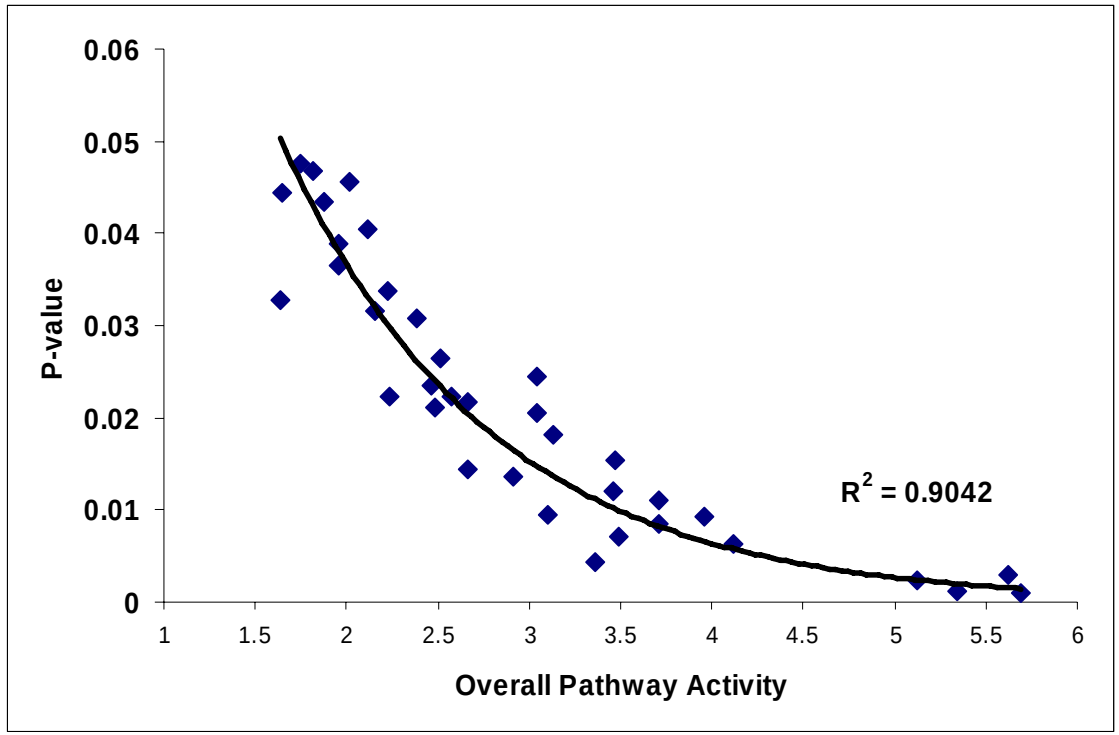
Significant pathways



Significance vs activity

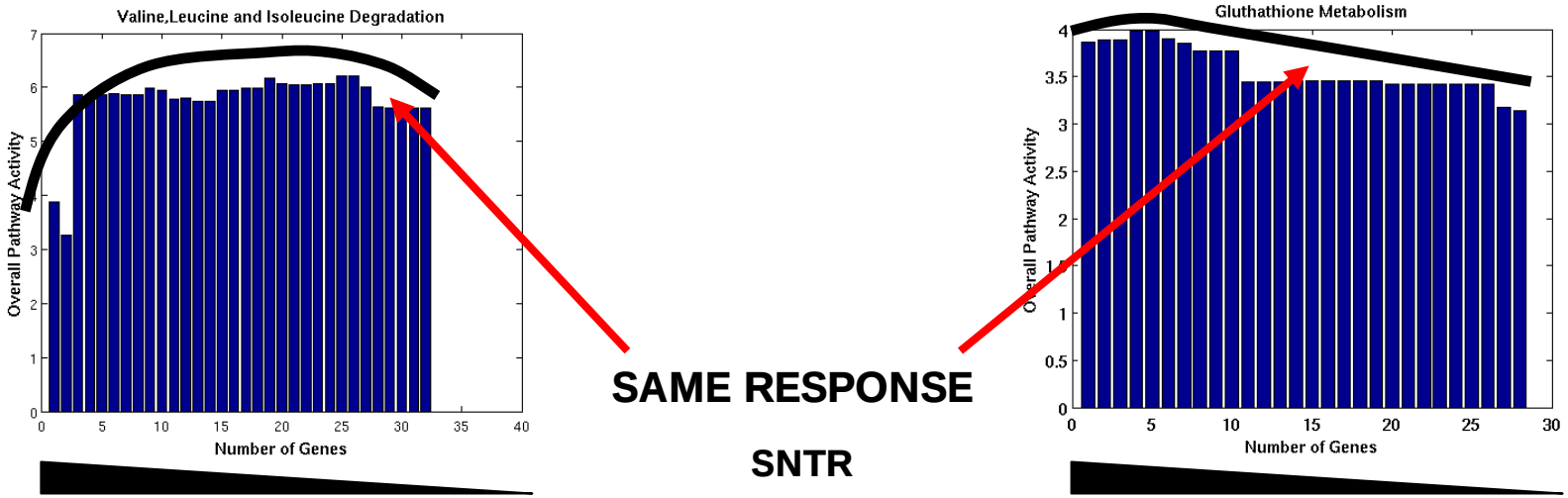


Does this particular group of genes' expression mean anything?



The higher the overall pathway activity, the lower is the p-value: this feature allowed us to rank and compare based on the overall pathway activity.

Pathway activity and informative genes

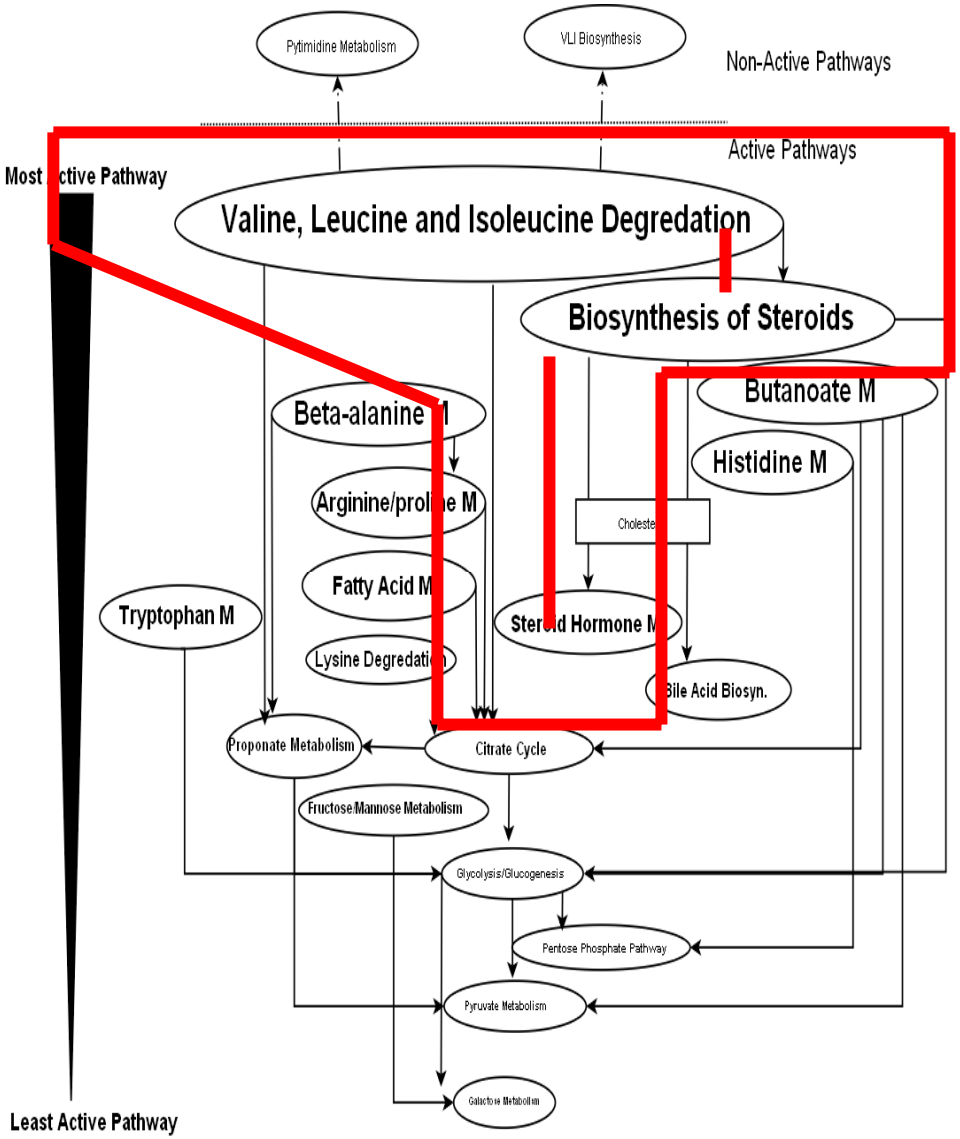


STNR- Ranked probe sets → Discriminating genes

Pre-defined set of genes, pathways → Differentially expressed pathways

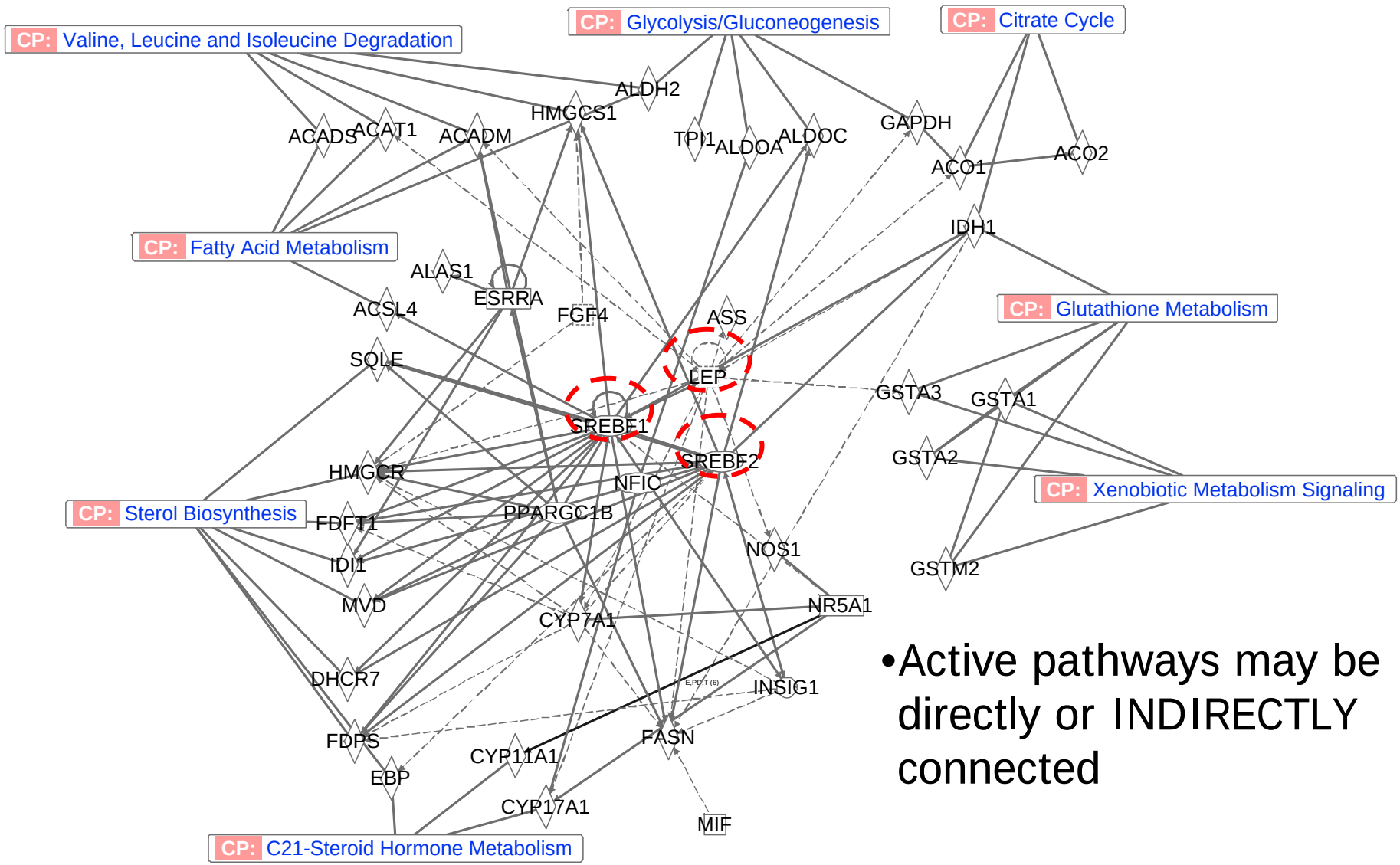
Discriminating genes in active pathways
"Informative genes"

Networks of interacting pathways



- The active pathways are connected via metabolites
- By tracking active pathways based on level of activity one can identify a putative sequence:
  VLI degradation → Bios. of Steroids → Sterol Biosynthesis

Gene interaction networks

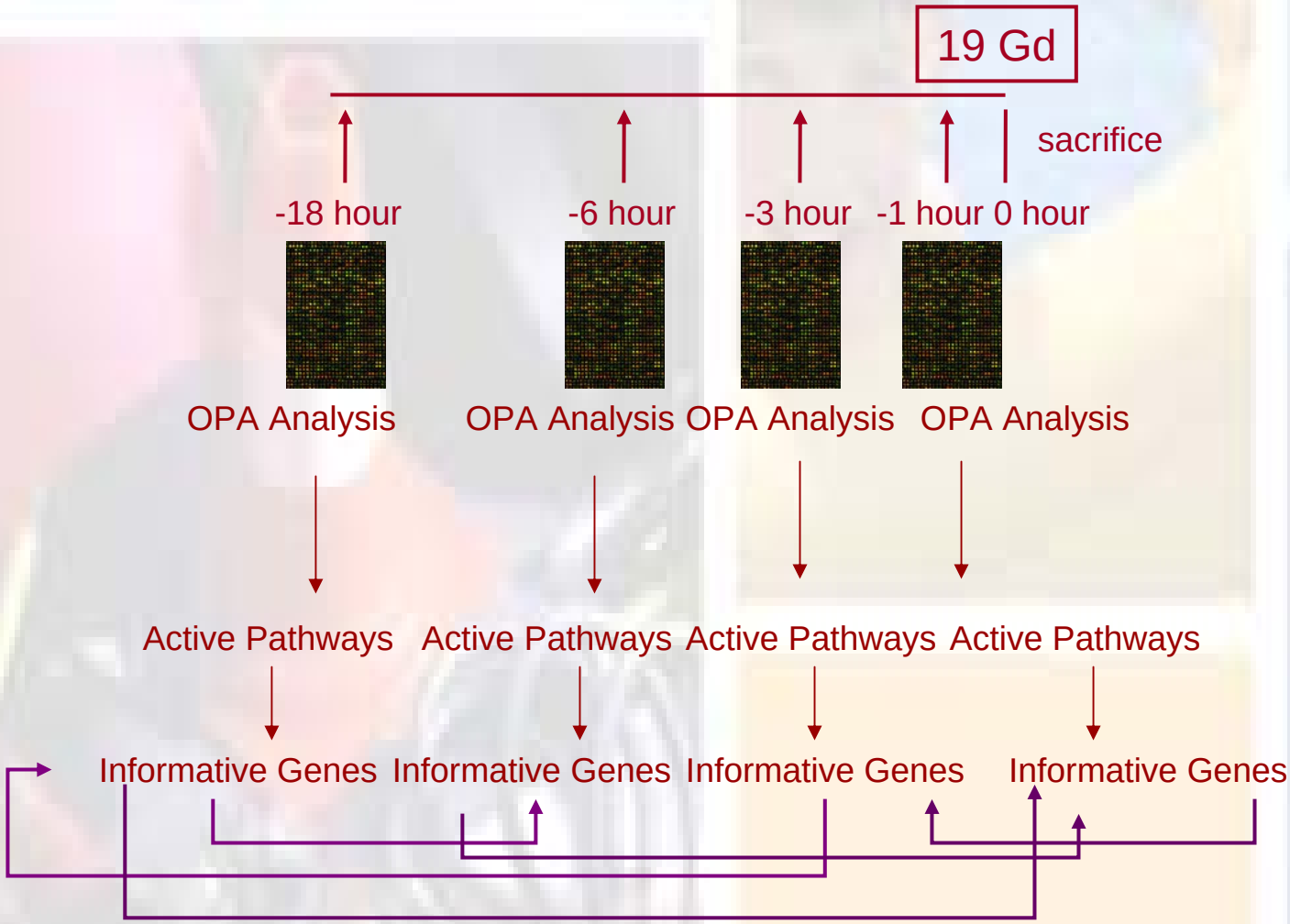


- Active pathways may be directly or INDIRECTLY connected

Effects of different dose durations

- Evaluate the implications of difference in duration exposure to DBP
- All animals sacrificed at same time point, but were exposed to DBP for different periods
- What kind of changes in gene expression are observed in the context of active pathways

Integrating analyses over time



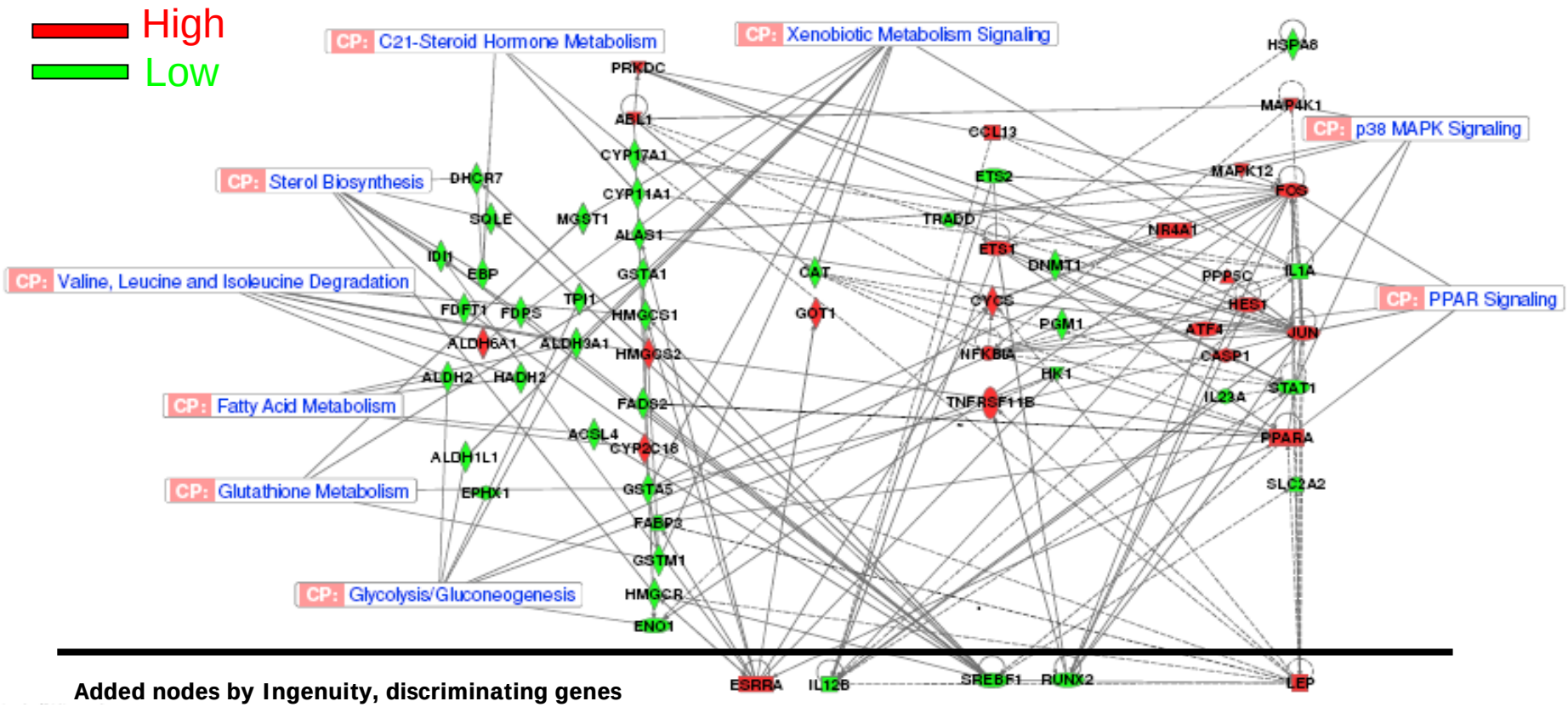
Localization of active pathways

− 18 Hour	− 6 Hour	− 3 Hour	−1 Hour
Sulfur metabolism	Amyotrophic lateral sclerosis ALS	1- and 2-Methylnaphthalene degradation	Maturity onset diabetes of the young
Phenylalanine, tyrsine and tryptophan biosynthesis	Glutamate metabolism	Dorso-ventral axis formation	Heparan sulfate biosynthesis
VLI Degradation		Methionine pathway	Hedghehog signaling pathway
Biosynthesis of steroids		Ascorbate and aldarate metabolism	Jak-Stat signaling pathway
Glycerine, Serine, threonine metabolism		Glycerophospholipid metabolism	Aminoacyl-tRNA biosynthesis
Ubiquinone biosynthesis		Starch (Glycogen) metabolism	N-Glycan biosynthesis
Fructose and mannose metabolism		Apoptosis	Inositol Phosphate metabolism
Fatty acid metabolism		Cytokine-Cytokine receptor interaction	Regulation of autophagy
Cell cycle			MAPK signaling pathway
Purine Metabolism			PPAR signaling pathway
Linoleic acid metabolism			
C21-Steroid hormone metabolism			
One carbon pool by folate			
Metabolism of xenabiotics of cytochrome P450			

Metabolic Pathways

Signaling Pathways

Gene interaction network



Dose Durations - 18 hour | - 6 hour | - 3 hour | - 1 hour

Summary

- Toxicogenomic data and methods provide the potential for elucidating toxicity mechanisms
- Focus is on combining expression and pathway data, as opposed to interpreting expressed genes independently
- For a pathway activity analysis, one should consider all genes in a given pathway
- Preliminary analysis is promising: however, in order to properly assess developmental effects of DBP more appropriate time-course studies need to be defined

Current work

- Model Improvement
  - How to better assess the concept of “discriminating genes” in the context of pathway activity
  - How to best represent and analyze time-course data
- Cross-Species Extrapolation
  - Pathway Topology Similarities
  - Protein Sequence

References

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5) Shultz V.D., Phillips S., Sar M., Foster P.M., Gaido K. Altered gene profiles in fetal rat testes after in utero exposure to di(n-butyl) phthalate. Toxicol Sci, 2001. 64(2): p. 233-42.

6) Barlow N.J., Phillips S.L., Wallace D.G., Sar M., Gaido K.W., Foster P.M., Quantitative changes in gene expression in fetal rat testes following exposure to di(n-butyl) phthalate. Toxicol Sci, 2003. 73(2): p. 431-41.

7) Liu K., Lehmann K.P., Sar M., Young S.S., Gaido K.W., Gene expression profiling following in utero exposure to phthalate esters reveals new gene targets in the etiology of testicular dysgenesis. Biol Reprod, 2005. 73(1): p. 180-92.

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10) Tomfohr, J., Lu J., Kepler T.B., Pathway level analysis of gene expression using singular value decomposition. BMC Bioinformatics, 2005. 6: p. 225