



Biological Properties in Genes for Low-Dose Regulation of the Embryonic Transcriptome

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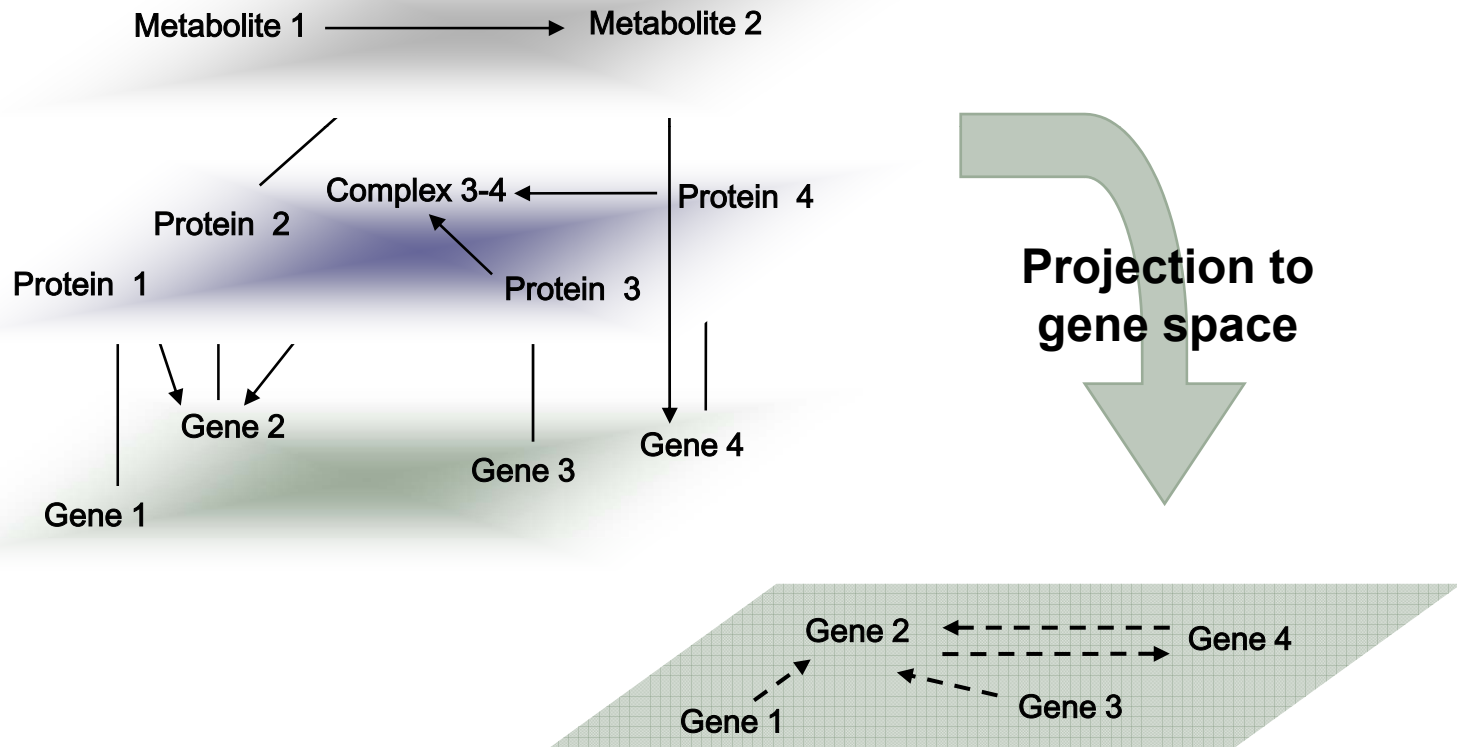
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Regulation of the embryonic transcriptome

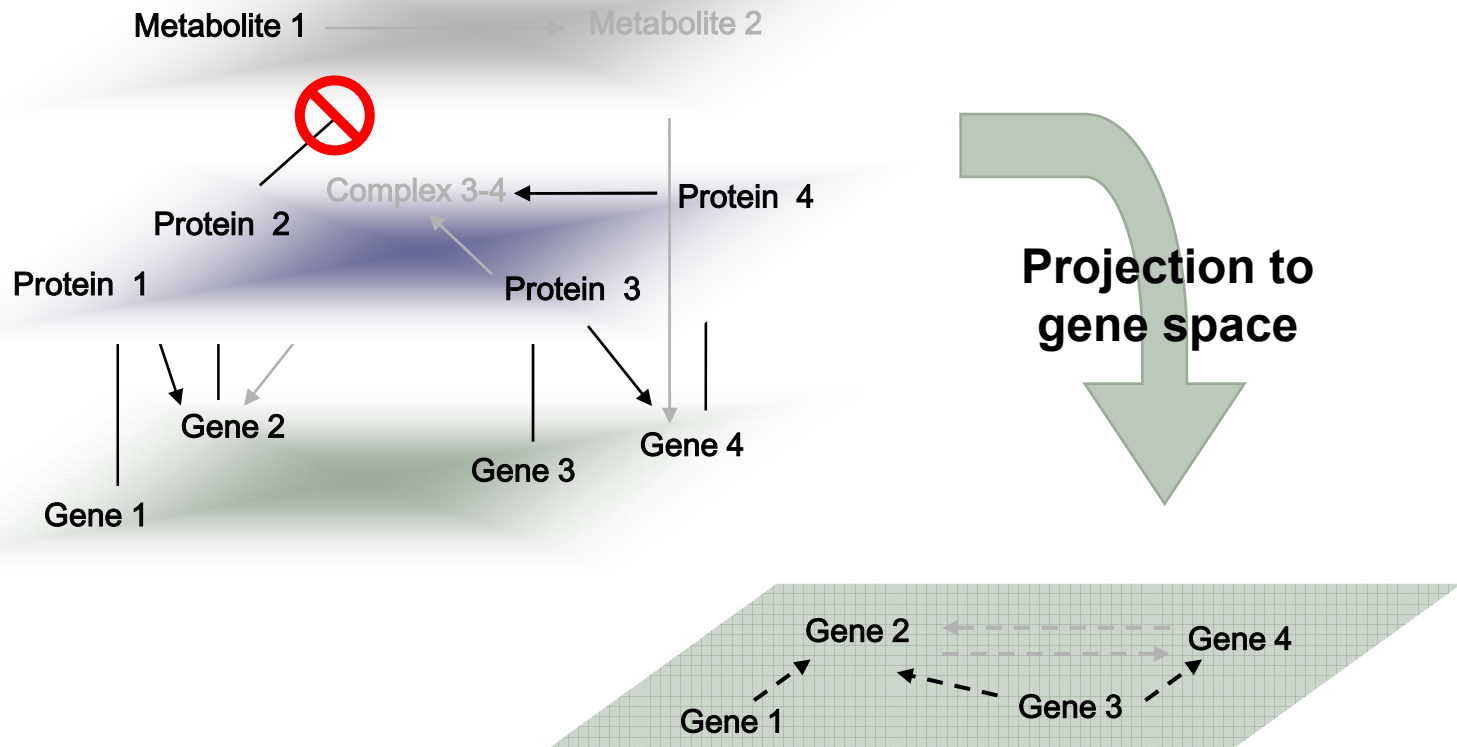


- ❖ **Premise:** chemical-induced adverse development (teratogenesis) is a threshold phenomenon
- ❖ **Hypothesis:** clinical phenotype (malformations) can be modeled from expression phenotype (transcriptome)
- ❖ **Goal:** characterize dose-response spectrum at the genomic level using microarray-based analysis

Gene networks as a projection of the integrated system



Gene networks as a projection of the integrated system - toxicity

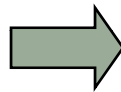
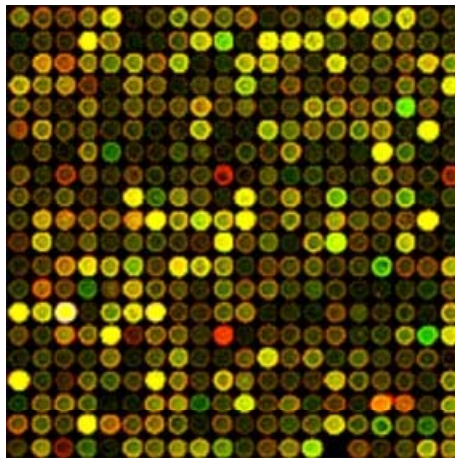


Practical and theoretical applications:



- ❖ functional annotation of genome-level response across the dose-response spectrum
- ❖ find intersect between programmed (genetic) and regulative (adaptive) pathways in development
- ❖ distinguish adaptive (homeostasis) from adverse (pathogenesis) processes
- ❖ computational systems models for gene regulatory networks that govern these state transitions

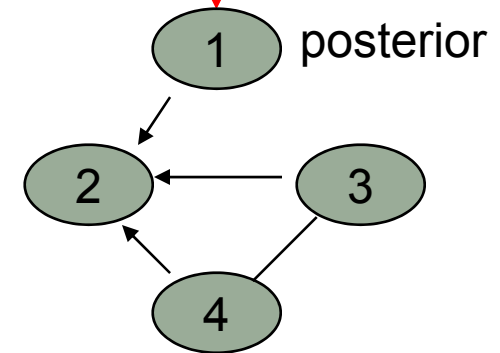
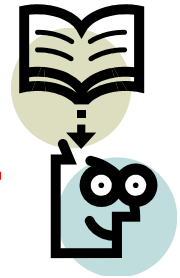
How to portray a 'bionetwork'



	116_at	1178_at	134_at
N01	2.6754	2.4320	3.6055
N02	3.2363	2.7115	4.5146
N03	3.2884	2.8985	4.1603
N04	3.1319	2.6948	4.3121
N05	3.3561	2.8743	4.1642
T01	2.7913	2.5813	3.5937
T02	3.2850	2.6469	4.0875
T03	3.1136	2.6666	3.9783
T04	2.7141	2.4659	3.3916
T05	3.0619	2.6139	3.9630

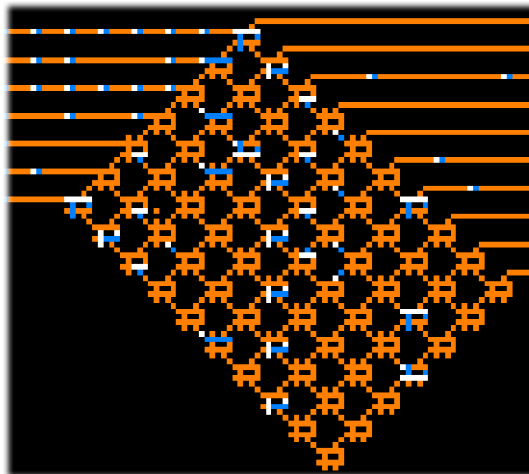
data

knowledge



computational
systems model

integrated
biological
response



- **network** structure & topology models
- quantitative knowledge of **interactions**
- quantized local and temporal **states**

Example 1: genomic response to maternal alcohol consumption during pregnancy



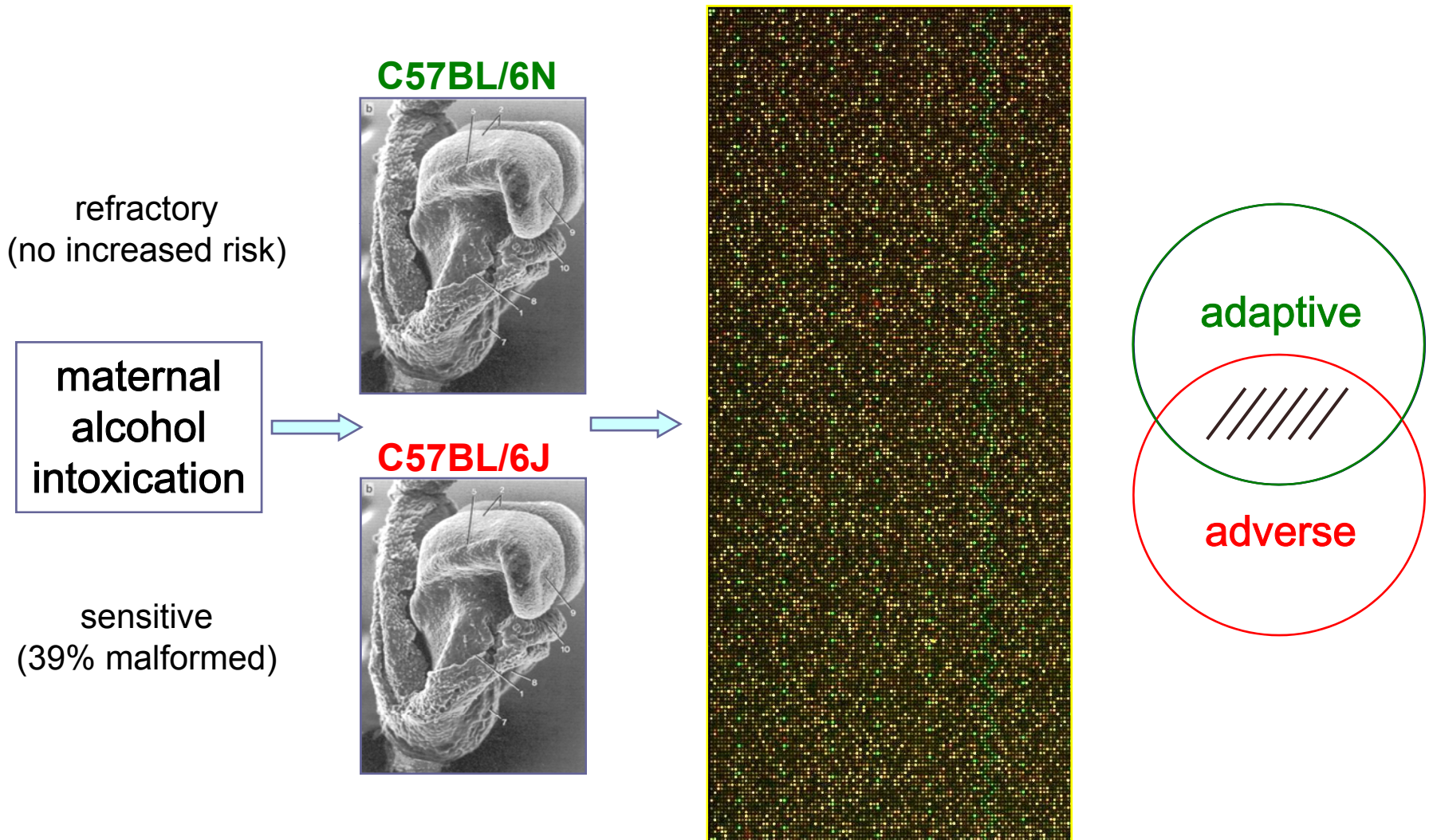
- 13% mothers drink during pregnancy
- 1% of babies damaged by alcohol
- Fetal Alcohol Syndrome (FAS)



murine FAS

Fetal Alcohol Syndrome (FAS)

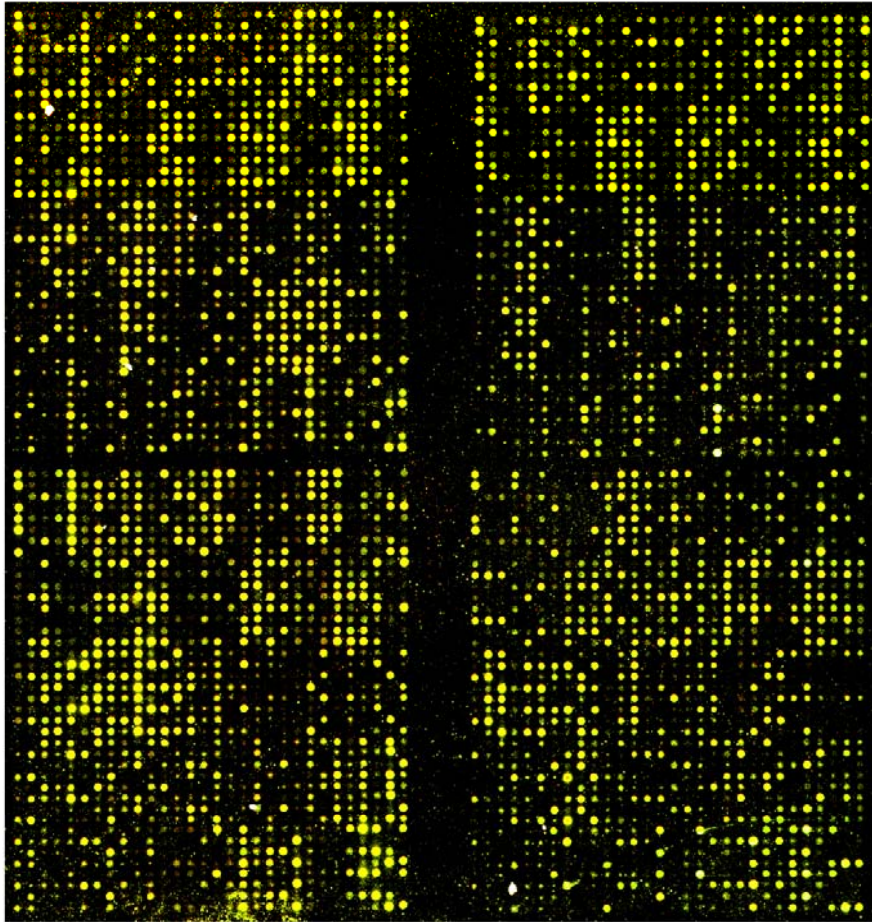
ethanol given to mice on gestation day 8



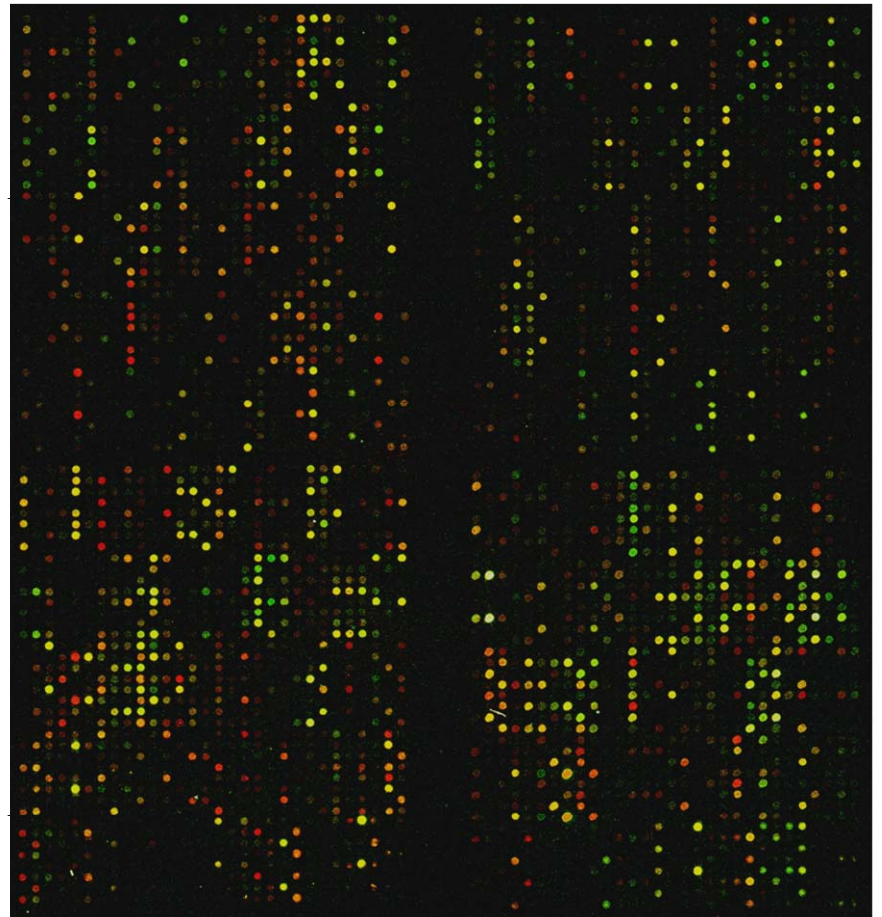
Cranial neural folds:

day 8 mouse embryos (3rd week human gestation)

normal embryos (green / red)

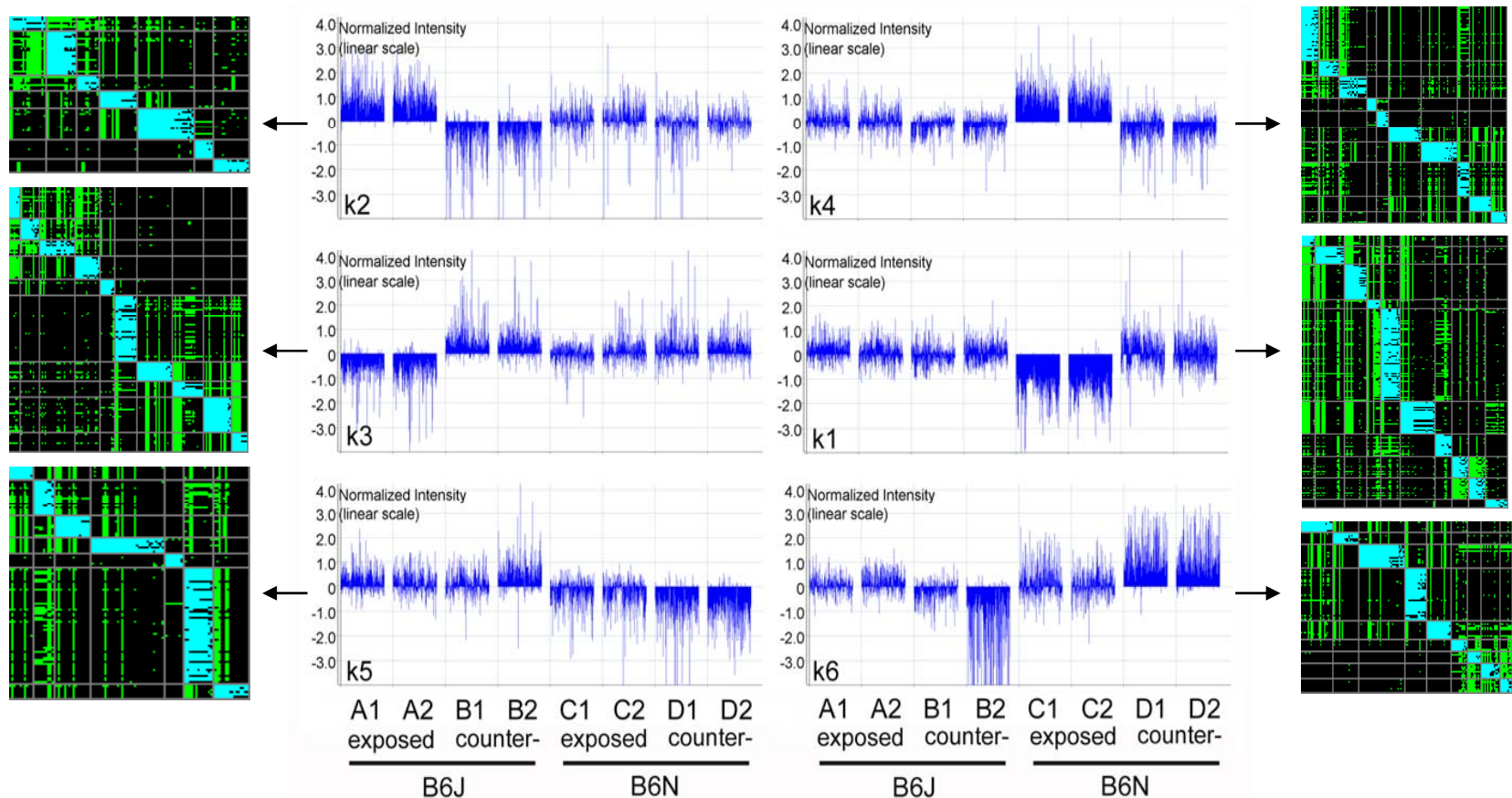


EtOH / control embryos (green / red)

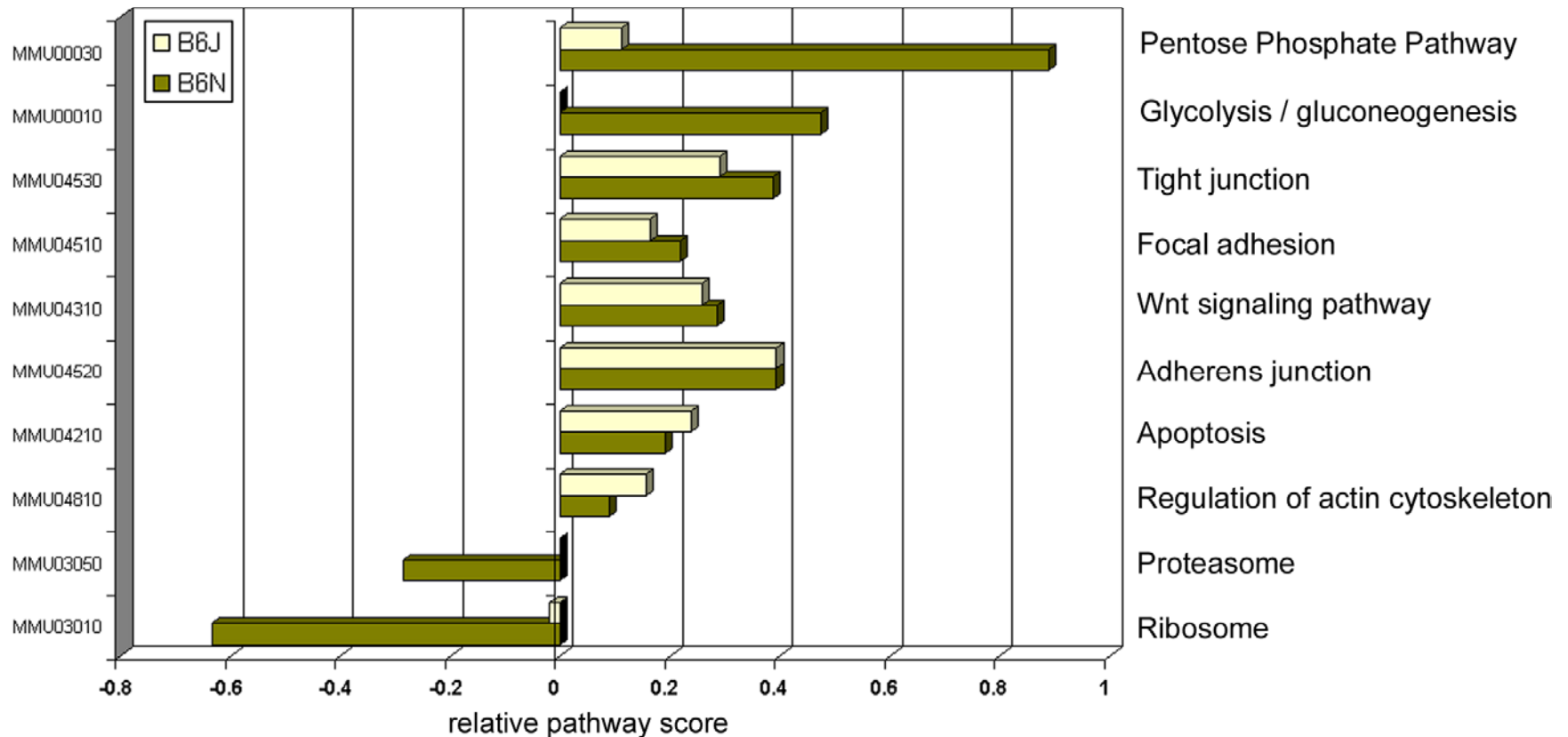


Functional mapping: 2906 genes in early embryos altered by maternal alcohol intoxication

Gene Ontology maps for 6 expression clusters (log2)



Global pathways reacting to EtOH



Source: Green et al. (2007) *Developmental Dynamics* 236: 613-631

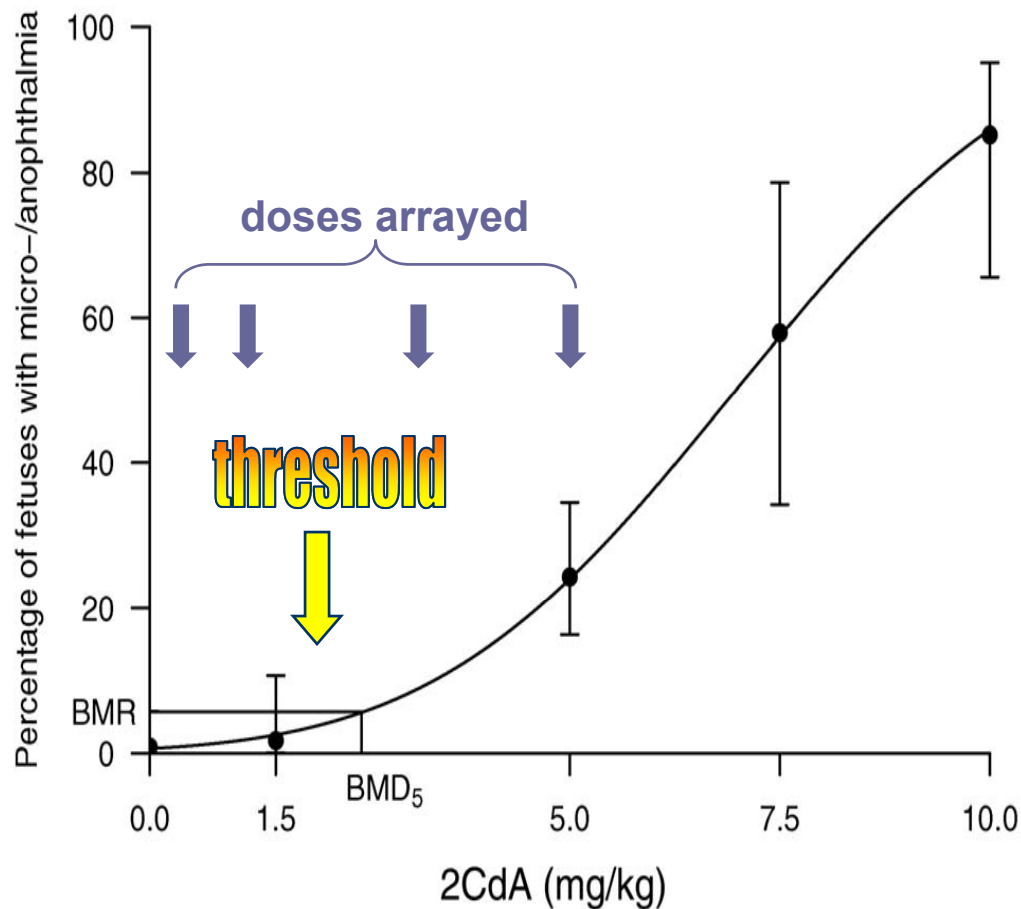
Receptor-mediated cell adhesion:

embryos reprogram cell **motility** (B6N) or **adhesivity** (B6J)

<i>Gene</i>	<i>B6J</i>	<i>B6N</i>	<i>B6J-B6N differential</i>
Procollagen 1 alpha 2	↑	↑	+ 0.12
Procollagen 6 alpha 2	↑	--	+ 0.74
Integrin alpha 3	↑	↓	+ 1.09
Integrin beta 3	↓	--	- 0.42
Caveolin-1	↑	--	+ 0.75
L1 cell adhesion molecule	--	↓	+ 0.20
Focal adhesion kinase (PTK2)	--	↓	+ 1.28
Ephrin b1	↑	↓	+ 1.38
Ephrin b2	--	↓	+ 1.61
Ephrin b3	↑	--	+ 0.88
Ephrin a5	↑	↓↓	+ 2.24
EPH receptor A2	--	↓	+ 0.68
EPH receptor A4	--	↓	+ 1.34
IGF-1	↑	--	+ 0.35
IGF-1 receptor	--	↓	+ 0.70

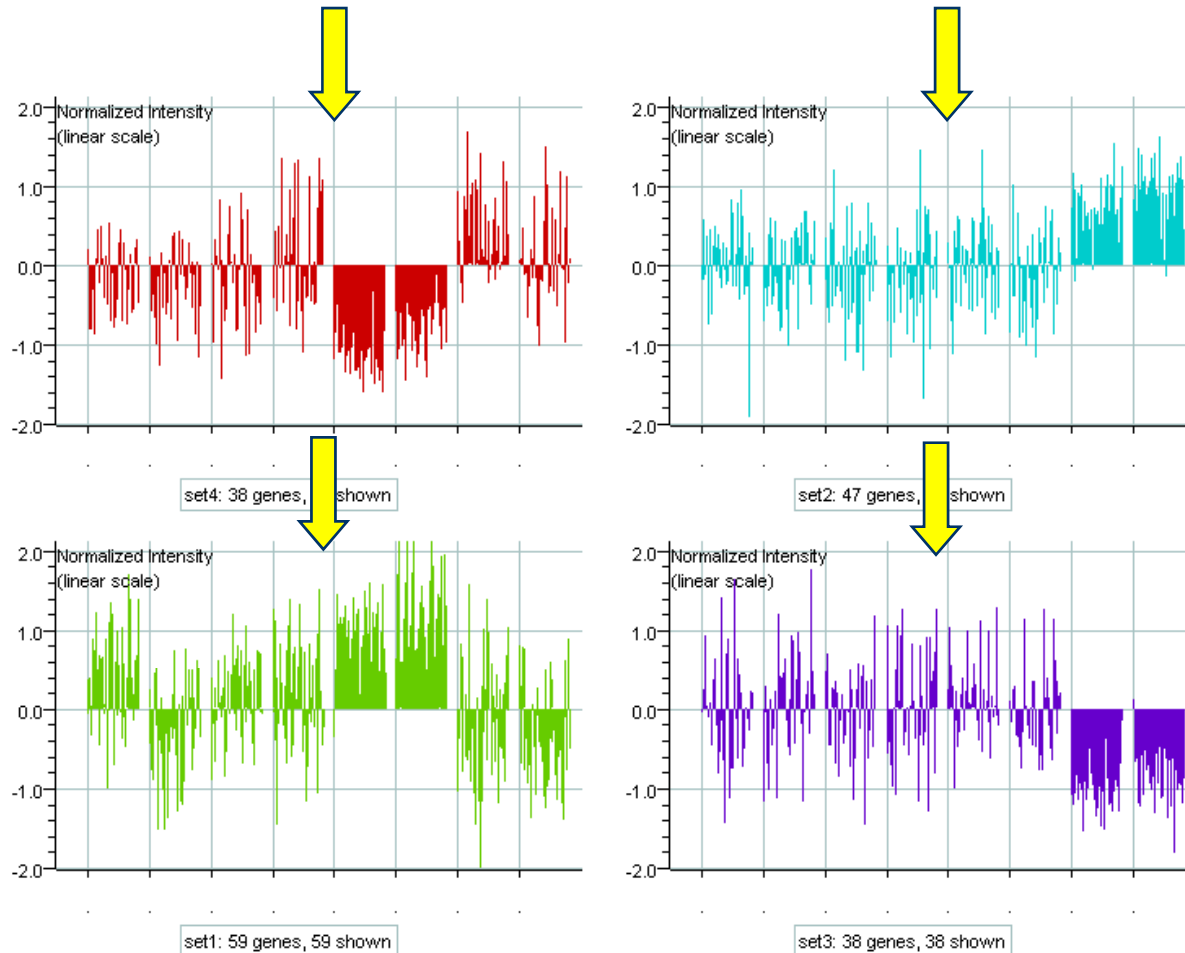
Subset of significantly altered genes in focal adhesion linked by functional classification and expression profile; up-/down regulation at threshold of 0.75-fold change. The differential is given in log(2) units (eg, 1 = two-fold change).

Example 2: microphthalmia induced with the ocular teratogen 2-chloro-2'-deoxyadenosine (2CdA)



Dose-response spectrum at 3.0 hr 2CdA

(182 genes grouped by k-means clustering)



Highest ranking biological themes: functional annotation of expression clusters 2 and 4

2. Genes up-regulated at BMD5

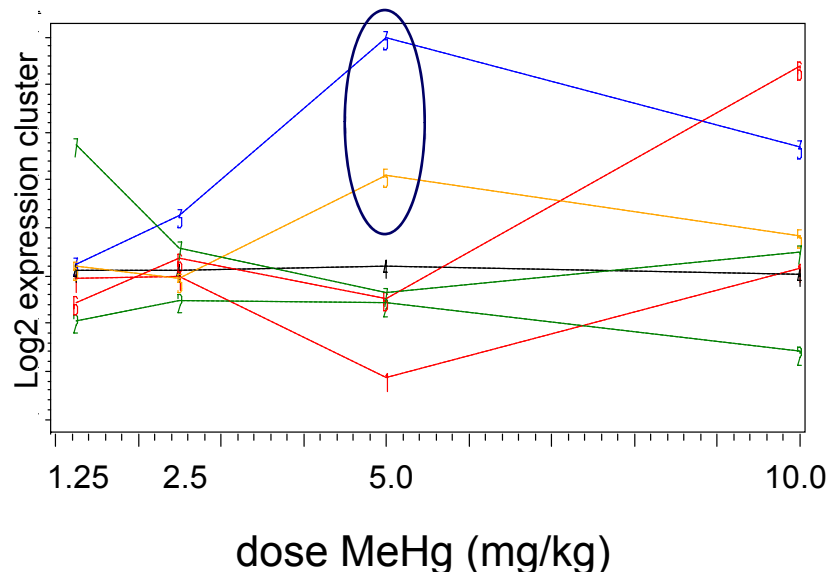
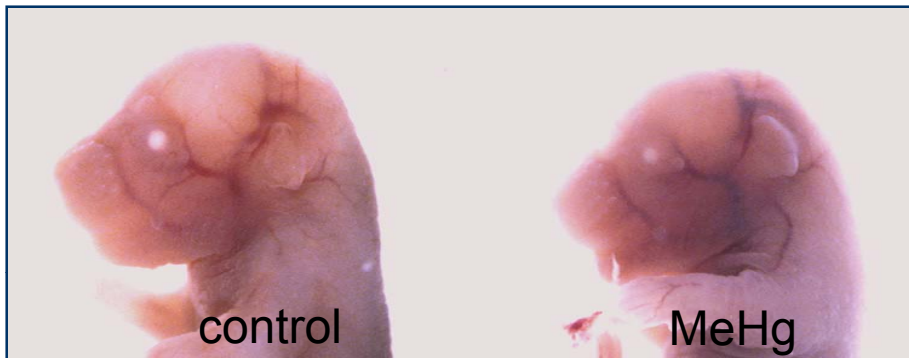
Annotation Cluster 1		Enrichment Score: 1.46
GOTERM_BP_ALL	protein catabolism	
GOTERM_BP_ALL	biopolymer catabolism	
GOTERM_BP_ALL	macromolecule catabolism	
Annotation Cluster 2		Enrichment Score: 1.44
GOTERM_BP_ALL	cell homeostasis	
GOTERM_BP_ALL	cation homeostasis	
GOTERM_BP_ALL	cell ion homeostasis	
GOTERM_BP_ALL	ion homeostasis	
GOTERM_BP_ALL	di-, tri-valent inorganic cation homeostasis	
GOTERM_BP_ALL	metal ion homeostasis	
Annotation Cluster 3		Enrichment Score: 1.19
GOTERM_BP_ALL	transport	
GOTERM_BP_ALL	establishment of localization	
GOTERM_BP_ALL	localization	

4. Genes down-regulated at BMD25

Annotation Cluster 1		Enrichment Score: 3.24
GOTERM_CC_ALL	cytosolic ribosome (sensu Eukaryota)	
GOTERM_CC_ALL	cytosolic large ribosomal subunit (sensu Eukaryota)	
GOTERM_CC_ALL	large ribosomal subunit	
Annotation Cluster 2		Enrichment Score: 2.82
GOTERM_MF_ALL	structural constituent of ribosome	
KEGG_PATHWAY	RIBOSOME	
GOTERM_CC_ALL	ribosome	
Annotation Cluster 3		Enrichment Score: 1.66
GOTERM_CC_ALL	nuclear lumen	
GOTERM_CC_ALL	organelle lumen	
GOTERM_CC_ALL	membrane-enclosed lumen	
Annotation Cluster 4		Enrichment Score: 1.34
GOTERM_BP_ALL	anti-apoptosis	
GOTERM_BP_ALL	negative regulation of apoptosis	
GOTERM_BP_ALL	negative regulation of programmed cell death	
GOTERM_BP_ALL	regulation of apoptosis	
GOTERM_BP_ALL	regulation of programmed cell death	

Example 3: Fetal Minamata Disease

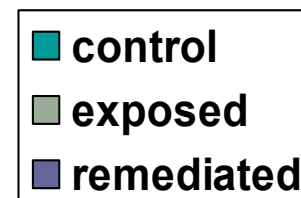
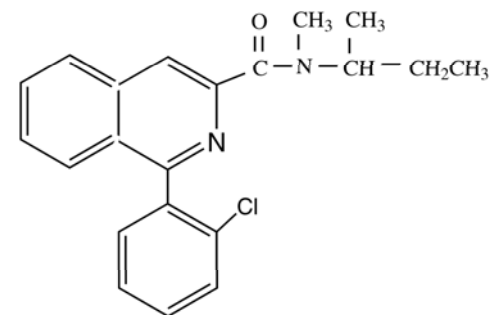
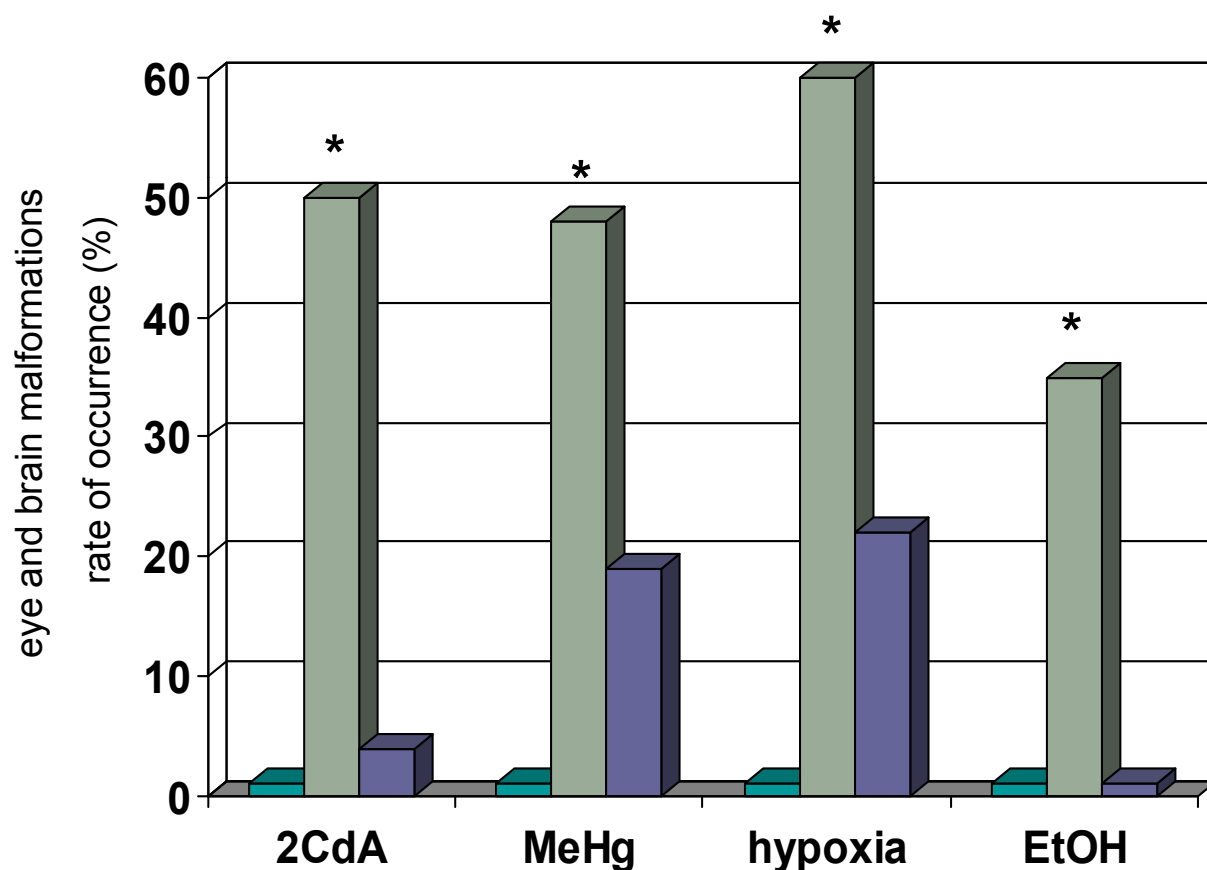
methylmercury (MeHg) on day 9



Annotation Cluster 1	Enrichment Score: 0.95
GOTERM_BP_ALL	intracellular signaling cascade
GOTERM_MF_ALL	signal transducer activity
GOTERM_BP_ALL	cell surface receptor linked signal transduction
GOTERM_BP_ALL	cell communication
GOTERM_BP_ALL	signal transduction
Annotation Cluster 2	Enrichment Score: 0.88
GOTERM_CC_ALL	intracellular
GOTERM_MF_ALL	nucleic acid binding
GOTERM_MF_ALL	DNA binding
Annotation Cluster 4	Enrichment Score: 0.79
GOTERM_MF_ALL	nucleic acid binding
GOTERM_MF_ALL	DNA binding
GOTERM_MF_ALL	protein binding
GOTERM_BP_ALL	nucleobase, nucleoside, nucleotide and nucleic acid metabolism
Annotation Cluster 8	Enrichment Score: 0.54
KEGG_PATHWAY	FOCAL ADHESION

Example 4: therapeutic intervention

maternal PK11195 treatment protects the embryo



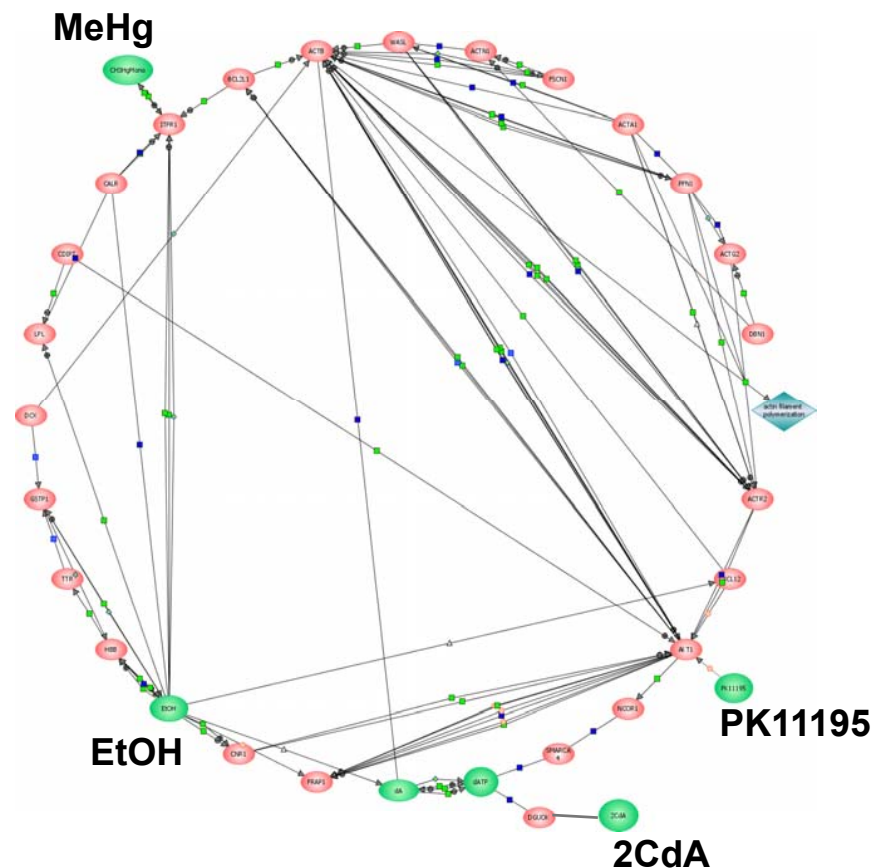
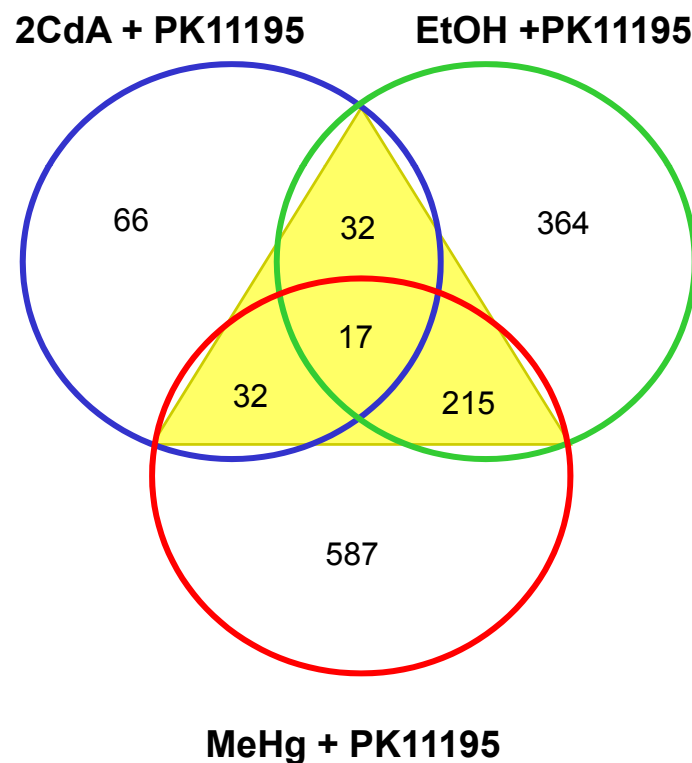
KEGG pathways of plasticity:

PK11195 responsive pathways in the early embryo

KEGG	PATHWAY	LIST	P value
HSA03010	RIBOSOME	55	2.52E-06
HSA04510	FOCAL ADHESION	41	0.010225
HSA04020	CALCIUM SIGNALING PATHWAY	36	0.006316
HSA04910	INSULIN SIGNALING PATHWAY	27	0.034363
HSA04070	PHOSPHATIDYLINOSITOL SIGNALING SYSTEM	23	0.005534
HSA04540	GAP JUNCTION	20	0.037796
HSA04730	LONG-TERM DEPRESSION	18	0.016329
HSA04520	ADHERENS JUNCTION	17	0.045554
HSA00010	GLYCOLYSIS / GLUCONEOGENESIS	16	0.011854
HSA04720	LONG-TERM POTENTIATION	15	0.029993
HSA03050	PROTEASOME	12	0.003279
HSA04930	TYPE II DIABETES MELLITUS	12	0.028197

'kernel' of an adaptive network:

primary hubs = ITPR1, FRAP1, AKT1 and ACTB



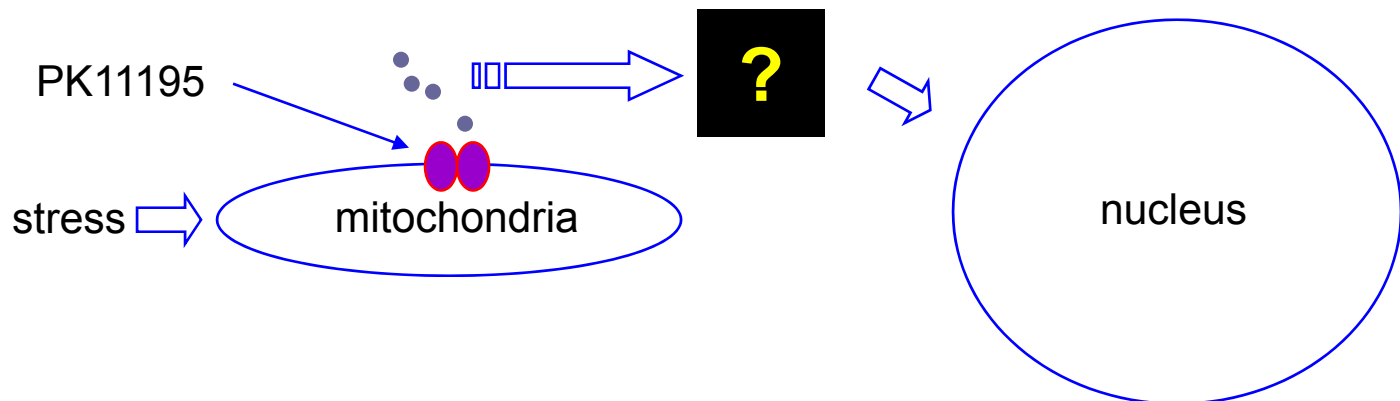
Summary



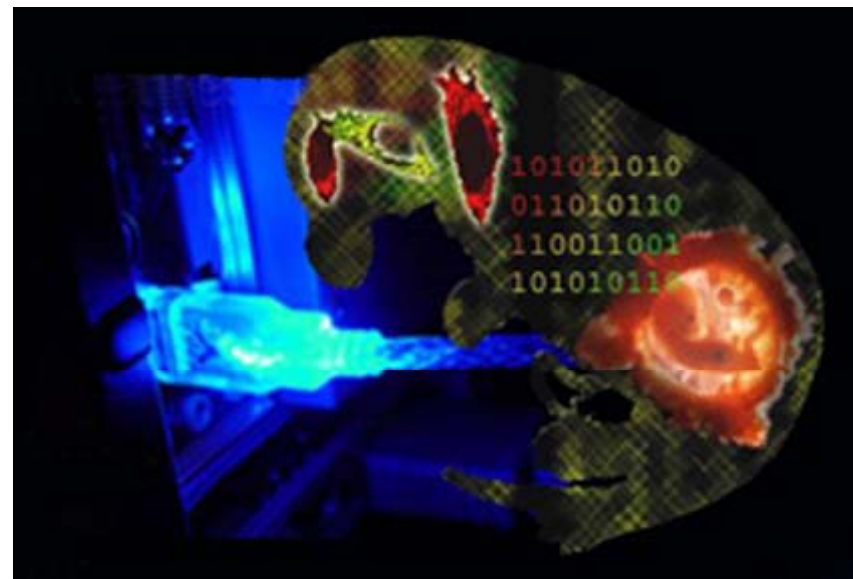
- ❖ Genomic response varied across dose-response spectrum: different genes, different programs
- ❖ Clinical phenotype (malformations) cannot be simply modeled from expression phenotype (transcriptome)
- ❖ Low-dose adaptive response should be considered in biosystems modeling efforts

Model for hormesis

- ❖ PK11195 stimulates an adaptive network that renders embryos less susceptible to teratogenesis
- ❖ It's receptor (TSPO) suggests a mechanism and a druggable target for 'hormesis'



Acknowledgements



<http://systemsanalysis.louisville.edu>

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