Comparative toxicogenomic analyses to elucidate the mechanisms of toxicity of SRP chemicals

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Superfund Research Program

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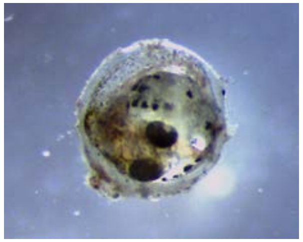
K.C. Donnelly Externship Award Supplement

Ecotoxicogenomics



- Aim to identify molecular fingerprints for environmental contaminants and predict adverse outcomes
- New sequencing technologies facilitate the use of non model/ ecologically relevant species
- Research questions
  - *Can we extrapolate the transcriptomic results of one species to others?*
  - *Are toxicity mechanisms well conserved or species-specific?*

KC Donnelly externship

UF | UNIVERSITY of FLORIDA
Dr Nancy Denslow

University of California Berkeley
Dr Christopher Vulpe

EST sequences assembled and annotated to yield over 10,000 sequences

↓

Microarrays
→ Genes differentially expressed

Library of 4,600 yeast strains, each lacking a single non-essential gene

↓

Parallel deletion assay
→ Genes required for growth

Outline

- Superfund chemicals investigated
- Methods for gene expression profiling
  - Oligonucleotides microarrays
  - Parallel deletion assay
- Results of comparative analyses
- Conclusions

SRP contaminants

- **Toxaphene** – organochlorine insecticide
  - Used on cotton, vegetables and livestock
  - Banned in 1990 in the US
  - Risks of thyroid problems, kidney and liver toxicity
- **Cadmium** – heavy metal
  - Natural and anthropogenic emission
  - Toxicity includes bone, kidney, lungs and liver diseases
- **Trichloroethylene** (TCE) – chlorinated hydrocarbon
  - Used as industrial solvent (degreaser for metal parts)
  - Reproductive and developmental effects in humans

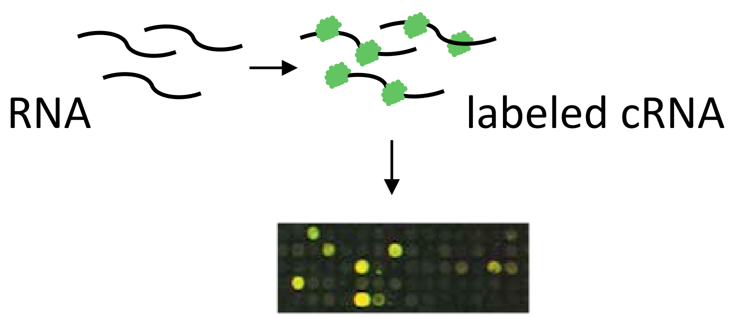
Gene expression profiling

<u>Microarrays</u>

1. Fish exposure to SRP chemicals

2. Total RNA extraction from liver

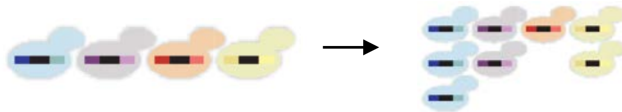
3. Cy3 labeling and hybridization



4. Data analysis

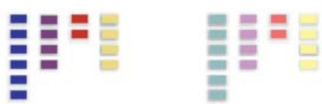
<u>Parallel deletion assay</u>

1. Grow yeast with SRP chemicals



2. Purify genomic DNA

3. PCR-amplify the barcodes



every strain has two tags

4. Hybridize “barcodes” to array

identifies strains affected by toxicant

5. Data analysis

Methods for comparative analysis

- Use human homologs and mammalian databases
- Find common genes affected by contaminants
- Identify common/similar biological processes affected
- Integrate the genomic data and characterize enriched sub-networks using Pathway Studio (Ariadne Genomics/Elsevier)

Toxaphene toxicity

- Different transcriptomic responses identified
- Bass- protein catabolic process and steroid binding
- Yeast- protein degradation, mitochondrial respiratory chain
- Transcription-related mechanisms impaired in both species
  - Differences in genomic profiles were to be expected as toxaphene is a neuroactive chemical that can disrupt the endocrine system.

Cadmium toxicity

Largemouth bass	Budding yeast
Heat shock response	
DNAJC7, HSPA14	DNAJC7, DNAJC3
Detoxification	
LDHB, AKR1B1	ALDH3A1, ALDH5A1, AKR1B1
Transcription	
POLR1C, POLR2C, TCEA1, TAF9, SAP30BP, PURB	SSN2 SAP30, SIN3A, HDA2
Transport (vesicle-mediated)	
TMEM39B CHMP7 SLC25A2	TMEM30A, TMEM165 CHMP1A, CHMP4B, CHMP3/5 SLC25A37

Sub-networks - cadmium

- Integration of yeast and fish data revealed that two sub-networks were affected by cadmium

bass genes = 10 yeast genes = 10

Protein folding

p = 0.002 protein folding

bass

2 genes 5 genes

Oxidative stress | Response to oxidative stress

16 genes 3 genes

yeast

p = 0.005 oxidative stress

p = 0.013 response ox. stress

TCE toxicity

Effects of cadmium common to both species:

- Genes: - cyclin B2
- Biological processes:
  - DNA replication
  - DNA recombination
  - DNA damage and DNA repair
  - Chromatin remodeling

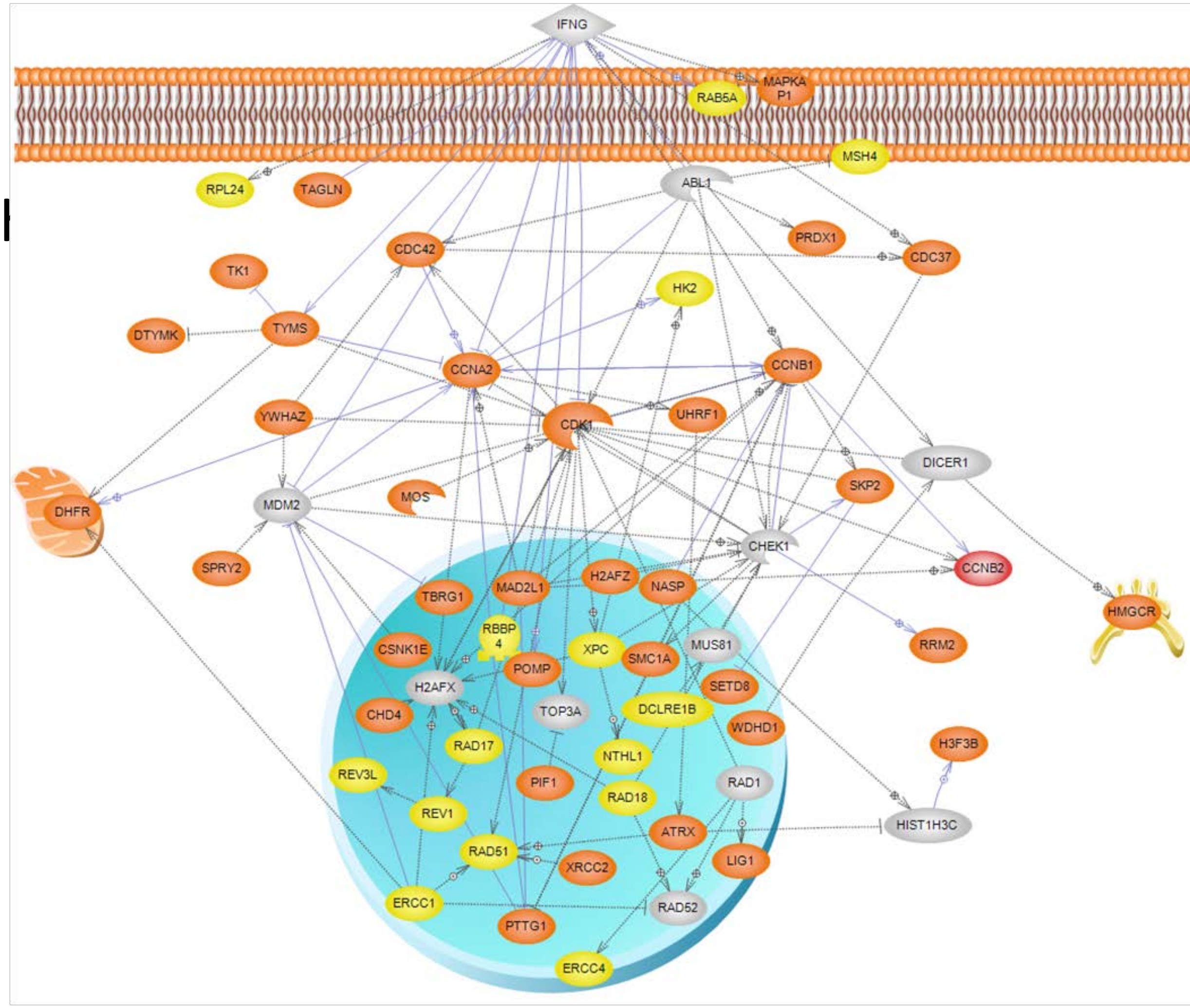
Sub-networks - TCE

fish gene
yeast gene
shared
unknown

Network enriched with terms related to :

- DNA repair,
- DNA replication
- response to DNA damage

All terms $p < 0.0001$



Conclusions

- Gene expression profiles characterized were species specific
- Common genes identified are involved in general toxicity- may not be suitable toxicity marker for specific contaminants
- Comparison of biological processes was more informative especially for chemicals affecting conserved mechanisms (e.g of TCE and DNA replication)
- It is more difficult to extrapolate the toxicity of chemicals with unique mode of actions (e.g EDCs)
- Integrating omics data for different organisms proved useful to identify sub-networks and pathways affected by SRP chemicals

Acknowledgments

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