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Biological Plausibility and Application to Risk Assessment: Human Relevance and Dose Response Analysis

Christopher J. Borgert, Ph.D.

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Biological Plausibility and Application to Risk Assessment: Human Relevance and Dose Response Analysis

Christopher J. Borgert[1,2], Patrick D. Guiney[3], George Casella[4], Kathleen T. Shiverick[5]

[1] Applied Pharmacology & Toxicology, Inc., Gainesville, FL
[2] Center for Environmental and Human Toxicology, Dept. of Physiological Sciences, University of Florida, Gainesville, FL
[3] S.C. Johnson & Son, Inc., Racine, WI
[4] Dept. of Statistics, University of Florida, Gainesville, FL
[5] Dept. of Pharmacology & Therapeutics, University of Florida, Gainesville, FL

Outline

Section 1: Philosophy

Section 2: Practice

Section 3: Pitfalls

Section 4: Progress

What is Biological Plausibility?

- " Biological plausibility means that the association is biologically believable." [testimony of eminent epidemiologist]
- *Consistent with Existing Knowledge*. Biological plausibility . . .depends upon existing knowledge about the mechanisms by which disease develops. . .[and] on the extent of scientific knowledge about the cellular and subcellular mechanisms through which the disease process works." (Reference Manual on Scientific Evidence - Epidemiology)

How is Biological Plausibility Used?

(f) Biological plausibility. An inference of causality tends to be strengthened by consistency with data from experimental studies or other sources demonstrating plausible biological mechanisms. A lack of mechanistic data, however, is not a reason to reject causality.

[Cancer Assessment Guidelines Section 2.2.1.7, page 2-14]

How is Biological Plausibility Evaluated?

To evaluate whether an hypothesized mode of action is operative, an analysis starts with . . . the hypothesized key events leading to cancer, and then weighing information to determine whether there is a causal relationship between these events and cancer formation, i.e., that the effects are critical for induction of tumors. It is not generally expected that the complete sequence will be known at the molecular level. Instead, empirical observations made at different levels of biological organization—biochemical, cellular, physiological, tissue, organ, and system—are analyzed.

[Cancer Assessment Guidelines Section 2.4.3, page 2-41.]

Causality: 1800's - 1900's

Henle-Koch, 1882
[In Scheutz & Poulsen, 1999]

1) Cause always present in disease
2) Cause NOT in other diseases
3) Cause is isolable from diseased individual
4) Isolated cause produces same disease in other individuals (animals)

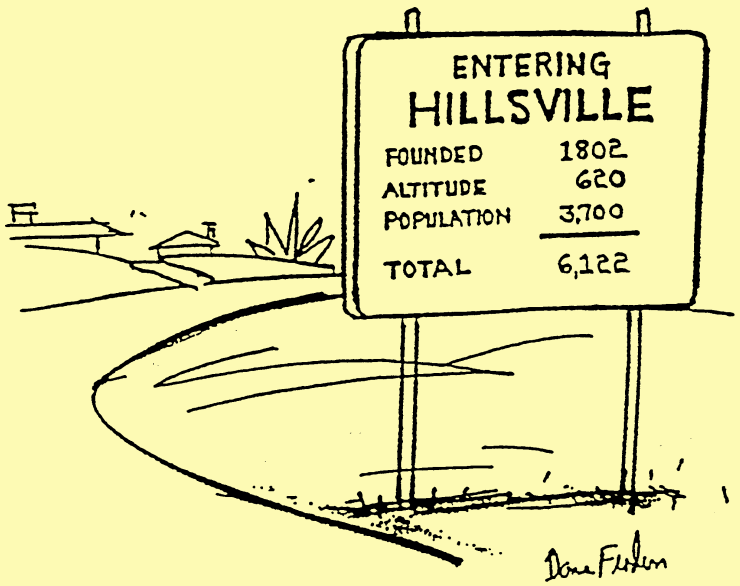
Hill, 1965

1) Strength
2) Consistency
3) Specificity
4) Temporality
5) Biological Gradient
6) Plausibility
7) Coherence
8) Experiment
9) Analogy

Causal Propositions vs. Causal Assessments

"Hill, Susser, and others recognize that these criteria do not define cause per se, but merely provide guidelines for assessing it. Nonetheless, the criteria are occasionally used by epidemiologists and clinical researchers as though they provide an operational definition of causality."

Kramer & Lane, 1992



Drawing by Dana Fradon: © 1997 The New Yorker Magazine, Inc.

"Cause" Requires the "Counterfactual"

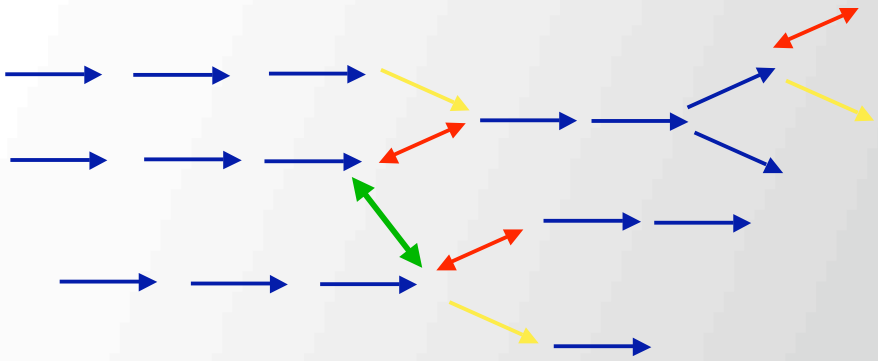
"*. . . an object followed by another . . .where, if the first object had not been, the second never had existed.*"

David Hume, 1748

"*A key innovation of this definition was that it pivoted on a clause of the form 'if C had not occurred, D would not have either,' where C and D are actually what occurred*."

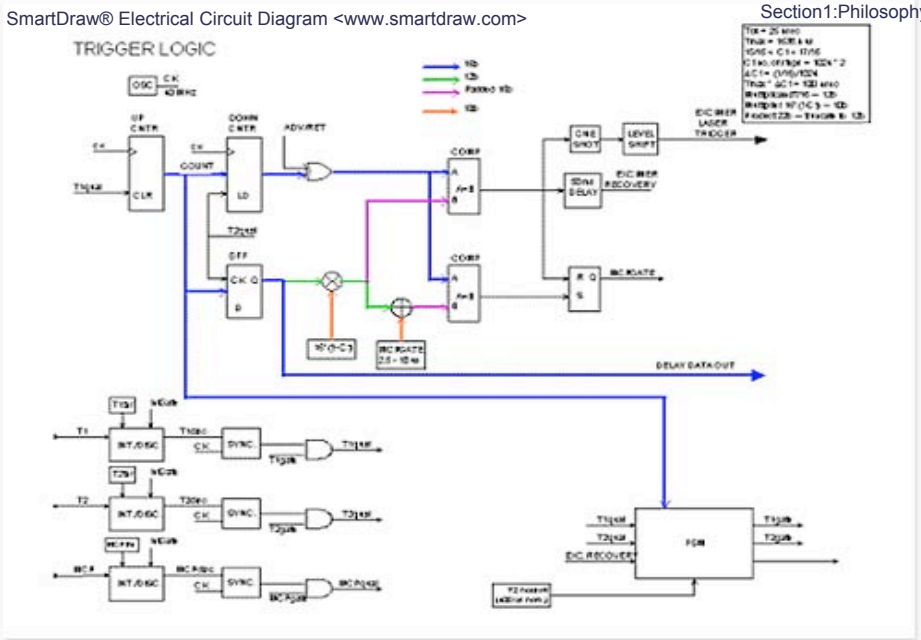
Maldonado & Greenland, 2002

Causal Chains
Linear Causalism

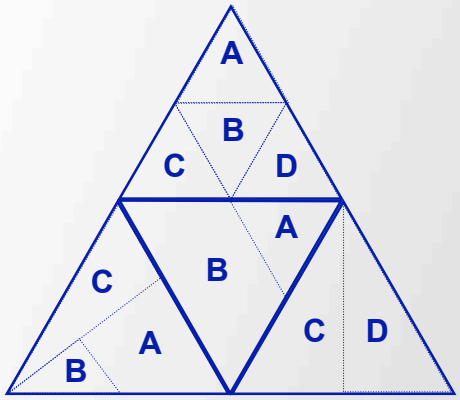


Attena, 1999

SmartDraw® Electrical Circuit Diagram <www.smartdraw.com>



Causal Mosaics
Risk Factor Probabilism

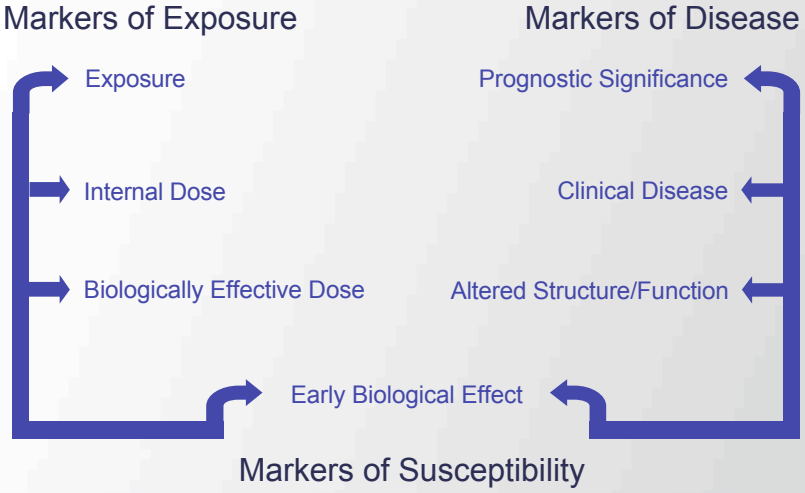


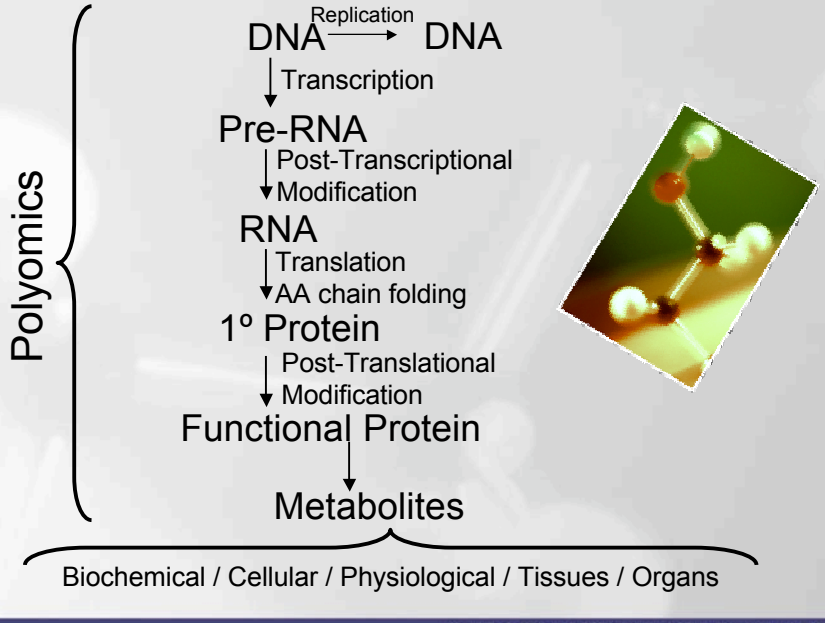
Scheutz & Poulsen, 1999

Omics Technologies *Promises*

- More sensitive biomarkers of exposure
- More precise biomarkers of effect
- Identify pre-toxicological changes
- Identify omic profiles associated with toxic responses
- Identify molecular mechanistic steps
- Elucidate complete toxicologic pathways
- Categorize chemicals by polyomic profiles
- Identify fundamentally similar modes of toxicity

Promises of Omic Technologies



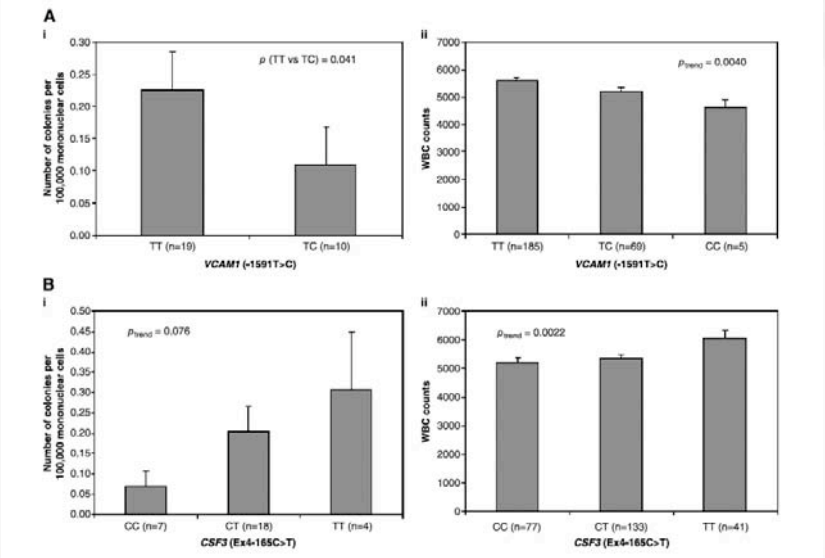


Chinese Shoe Worker Cohort

- 250 workers from two shoe factories in China
- Broad range of benzene exposure
- Benzene levels analyzed over 16 months
- Co-exposure and sources investigated by principal component analysis and mixed effects models
- Range = 0.2-50 ppm (highest in glue handlers)

Vermeulen *et al*., 2004. Ann. Occup. Hyg. 48(2):105-116.

Lan *et al*., 2005. Polymorphisms in cytokine and cellular adhesion molecule genes and susceptibility to hematotoxicity among workers exposed to benzene. Cancer Res. 65(20): 9574-81.



Chinese Shoe Worker Cohort

Proteomic Study Description

- Exposed: N=20 shoe workers - benzene levels monitored for 3 months prior to blood sample collection
- Control: N=20 clothing factory workers
- Blood serum proteins separated by pH, fractions applied to ProteinChip and analyzed by SELDI-TOF MS for mass/charge spectrum
- Created protein peak clusters and calculated p values for differences in peak intensity between exposed and control samples, those with $p<0.01$ were identified as potential biomarkers of interest (n=3)
- The three potential biomarkers were digested and analyzed by tandem MS for protein identification. Identification was confirmed by antibody ProteinChip analysis.

Vermeulen et al. 2005. PNAS 102(47):17041-17046.

Chinese Shoe Worker Cohort

Proteomic Study Results

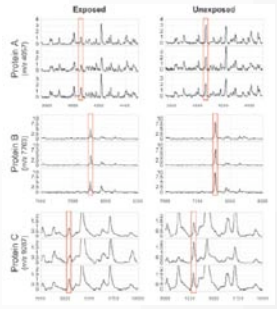


Figure 4. Differential protein expression - Physical property ProteinChip SELDI-TOF MS

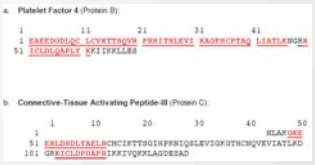


Figure 5. Protein identification Tandem MS

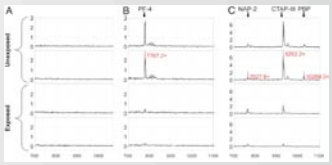


Figure 2. Confirmation of protein ID Antibody ProteinChip SELDI-TOF MS

Vermeulen et al. 2005. PNAS 102(47):17041-17046.

Chinese Shoe Worker Cohort

Metabolomic Study Description

- Exposed: N=250 shoe workers - benzene levels monitored by personal air samplers and urinary unmetabolized benzene
- Control: N=139 - benzene levels monitored by personal air samplers but measurements were below LOD so were estimated from urinary unmetabolized benzene
- Urine samples were analyzed by GC-MS for unmetabolized benzene and four benzene metabolites: *E,E*-muconic acid (MA), *S*-phenylmercapturic acid (SPMA), phenol (PH), catechol (CA), and hydroquinone (HQ).
- Median shoe worker benzene exposure = 1.2ppm (01017-88.9 ppm). Median control benzene exposure (est.) = 0.005ppm (0.002-0.007 ppm)
- As exposure level increased, level of each metabolite also increased
- As exposure level increased, ratio of metabolite:benzene decreased (except SPMA) due to saturation of CYP metabolism

Vermeulen et al. 2005. PNAS 102(47):17041-17046.

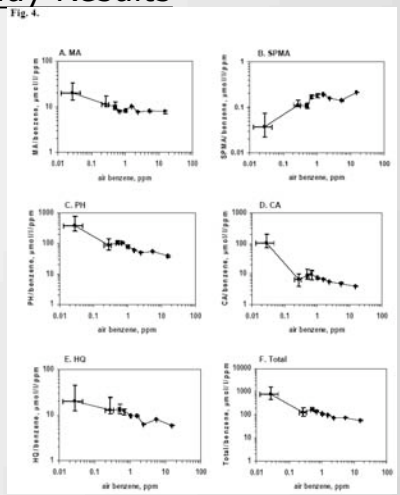
Chinese Shoe Worker Cohort

Metabolomic Study Results

Table II. Summary statistics for exposure to benzene and urinary analytes.

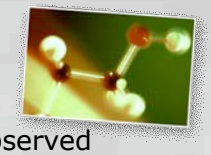
Variable	Control				Exposed			
	Female (n = 87)	Male (n = 52)	Nonsmokers (n = 100)	Smokers (n = 39)	Female (n = 164)	Male (n = 86)	Nonsmokers (n = 198)	Smokers (n = 52)
Air benzene[1]								
Median	3.40 ppb	3.71 ppb	3.09 ppb	6.07 ppb	1.28 ppm	1.05 ppm	1.18 ppm	1.18 ppm
Range	0.146 – 21.2 ppb	0.146–553 ppb	0.146 – 21.2 ppb	0.146 – 553 ppb	0.017 – 88.9 ppm	0.122 – 50.2 ppm	0.017 – 88.9 ppm	0.122 – 40.1 ppm
IQ range	1.70 – 6.80 ppb	2.15 – 9.71 ppb	1.70 – 5.91 ppb	2.56 – 17.6 ppb	0.523 – 4.21 ppm	0.572– 2.36 ppm	0.526– 3.34 ppm	0.588 – 3.02 ppm
p-value	0.273		0.003		0.588		0.089	
UBz (nmol/l)								
Median	1.48	1.59	1.36	2.47	283	216	267	197
Range	0.091–7.47	0.091–130	0.091–7.47	0.091–130	4.21–53900	19.4–42600	6.21–53900	19.4–41600
IQ range	0.799–2.75	0.973–3.74	0.797–2.41	1.25–4.32	87.3–1020	96.3–643	96.3 – 810	83.3–654
p-value	0.273		0.003		0.633		0.702	
MA (nmol/l)								
Median	1.06	1.09	1.06	1.11	13.5	10.3	12.3	12.4
Range	0.132–4.17	0.132–5.78	0.132–4.17	0.132–4.96	0.644–428	1.50–370	0.644–428	2.15–347
IQ range	0.540–1.75	0.774–1.72	0.543–1.70	0.803–1.74	4.35–34.5	3.39–22.4	4.13–28.5	4.22–28.4
p-value	0.565		0.384		0.272		0.472	
SPMA (nmol/l)								
Median	1.94	3.24	1.96	3.17	242	157	228	171
Range	0.591–88.4	0.591–68.1	0.591–88.4	0.591–68.1	1.50–29400	3.68–33000	1.50–29400	4.06–33000
IQ range	1.17–5.62	1.04–6.93	1.13–4.25	1.09–7.37	50.6–788	48.9–353	50.1–431	47.9 – 412
p-value	0.479		0.493		0.048		0.438	
PH (nmol/l)								
Median	40.4	53.2	41.3	56.3	171	154	158	155
Range	9.48–208	7.58–336	9.48–208	7.58–336	13.9–5740	41.8–4140	13.9–4140	41.8–3380
IQ range	45.0–103	28.8–82.0	43.7–89.6	29.0–98.5	103–310	86.6–190	100–261	84.6–313
p-value	0.117		0.415		0.038		0.191	
CA (nmol/l)								
Median	13.3	12.7	12.1	15.7	21.8	21.0	20.3	25.7
Range	2.30–61.1	2.45–46.6	2.30–61.1	2.45–46.6	3.50–420	4.20–438	3.50–438	7.08–348
IQ range	8.06–17.0	7.07–18.9	7.50–15.8	9.90–19.8	13.3–33.0	12.6–29.4	12.6–32.1	13.8–35.5
p-value	0.786		0.014		0.411		0.155	
HQ (nmol/l)								
Median	5.95	7.35	5.95	8.52	17.7	18.5	17.1	19.6
Range	1.56–39.1	1.52–41.7	1.46–39.1	1.52–41.7	2.28–427	3.26–341	2.28–427	3.76–275
IQ range	4.44–8.88	4.48–11.7	4.11–8.86	5.65–13.9	11.2–34.8	11.5–27.8	10.4–29.1	14.9–35.9
p-value	0.106		0.001		0.925		0.084	

Legend: IQ range - interquartile range, benzene - benzene in air, UBz - urinary benzene, MA - *E,E*- muconic acid, SPMA - *S*-phenylmercapturic acid, PH - phenol, CA - catechol, HQ - hydroquinone.
[1] Exposure to benzene for control subjects was predicted from the relationship between UBz and air exposure.



Kim et al. 2006. Carcinogenesis: Epub.

Reliability: Molecular Correlations



- Directly addressed the question of whether the observed changes in mRNA expression were also reflected at the level of protein abundance.
- DNA microarrays, quantitative proteomics (using isotope-coded affinity tag (ICAT) reagents, and tandem mass spectrometry MS/MS) were used in an integrated approach to directly compare protein abundance with mRNA expression levels in Yeast.
- As a whole, protein-abundance ratios were moderately correlated with their mRNA counterparts (r = 0.61)
- Underscores the importance of integrated mRNA- and protein-expression measurements.

Ideker *et al.,* 2001. Science 292(5518): 929.

Cytokine mRNA vs. Secreted Protein in RL 95-2 Human Uterine Endometrial Adenocarcinoma Cells Shiverick *et al*., unpublished data

mRNA

Control: *IL-1a = IL-1b>>IL-8*

BaP (10 uM)/TCDD (1 nM):

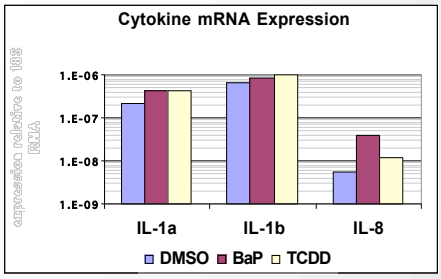
Increased IL-1a, IL-1B, IL-8

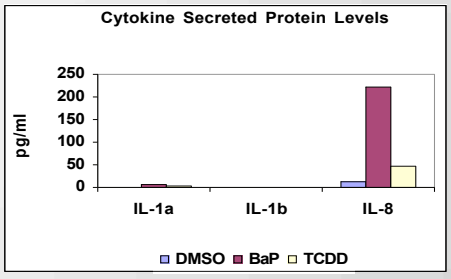
Secreted Protein

Control: *only IL-8 detected*

BaP/TCDD:

>>>> IL-8, slight > Il-1a





mRNA: Real-Time PCR using Taqman® Cytokine Gene Expression Plate

Protein in conditioned culture media: Quantikine ELISA Assay

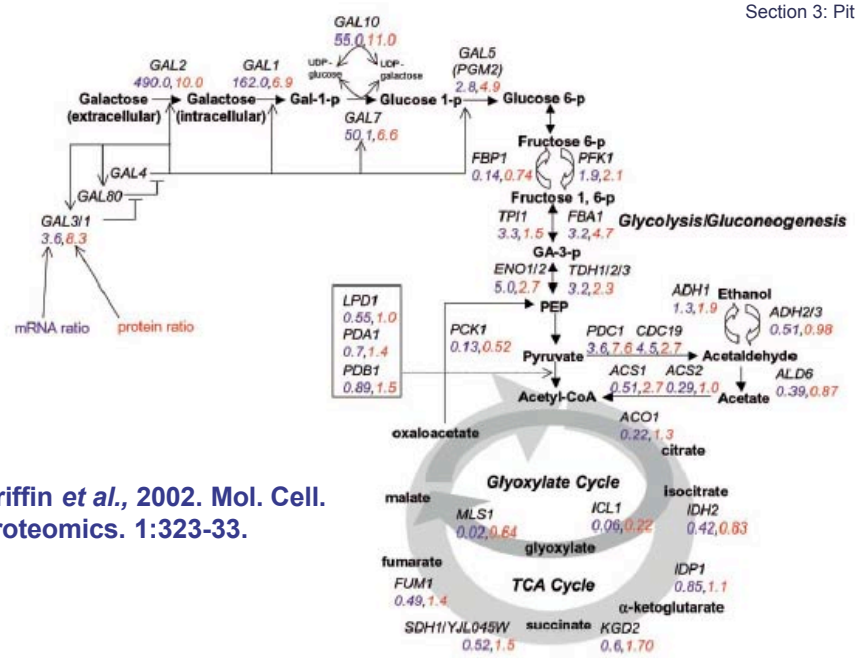
Genomics to Proteomics

- In similar fashion, protein levels *per se* may not be reflective of protein function or enzyme activity. Protein function and enzyme activity may depend on posttranslational modifications that are controlled by second messenger systems or other gene products not captured by an omic analysis of chemical effects.

- In order to probe how the polyomic level of biological organization is involved in a specific mode of toxic action, it will be necessary to design experiments that probe whether gene expression results in expression of functional proteins that are appropriately targeted to the correct intra/extracellular compartments.

Griffin *et al.,* 2002. Mol. Cell. Proteomics. 1:323-33.

Using an integrated genomic and proteomic approach, we have investigated the effects of carbon source perturbation on steady-state gene expression in the yeast Saccharomyces cerevisiae growing on either galactose or ethanol. ***For many genes, significant differences between the abundance ratio of the messenger RNA transcript and the corresponding protein product were observed. Insights into the perturbative effects on genes involved in respiration, energy generation, and protein synthesis were obtained that would not have been apparent from measurements made at either the messenger RNA or protein level alone***, illustrating the power of integrating different types of data obtained from the same sample for the comprehensive characterization of biological systems and processes.



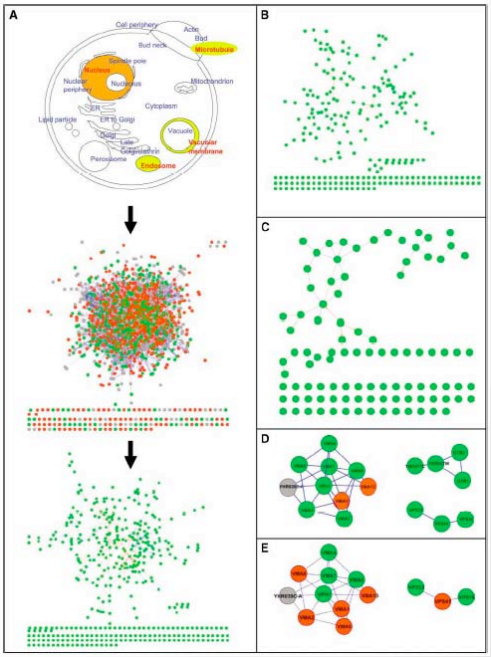
Griffin *et al.*, 2002. Mol. Cell. Proteomics. 1:323-33.

Haugen *et al., 2004*. Integrating phenotypic and expression profiles to map arsenic-response networks. Genome Biol. 5:R95

The cellular effects of arsenic were profiled by gene expression and sensitivity phenotypes in S. cerevisiae, and mapped to a metabolic network composed of all known biochemical reactions in yeast, as well as the yeast network of 20,985 protein-protein/protein-DNA interactions.

Contrary to the gene-expression analyses, the phenotypic-profiling data mapped to the metabolic network.

Arsenic is likely to channel sulfur into glutathione for detoxification, leads to indirect oxidative stress by depleting glutathione pools, and alters protein turnover via arsenation of sulfhydryl groups on proteins.



Begley *et al*., 2004.
Hot spots for modulating toxicity identified by genomic phenotyping and localization mapping.
Mol Cell. 16:117-25.

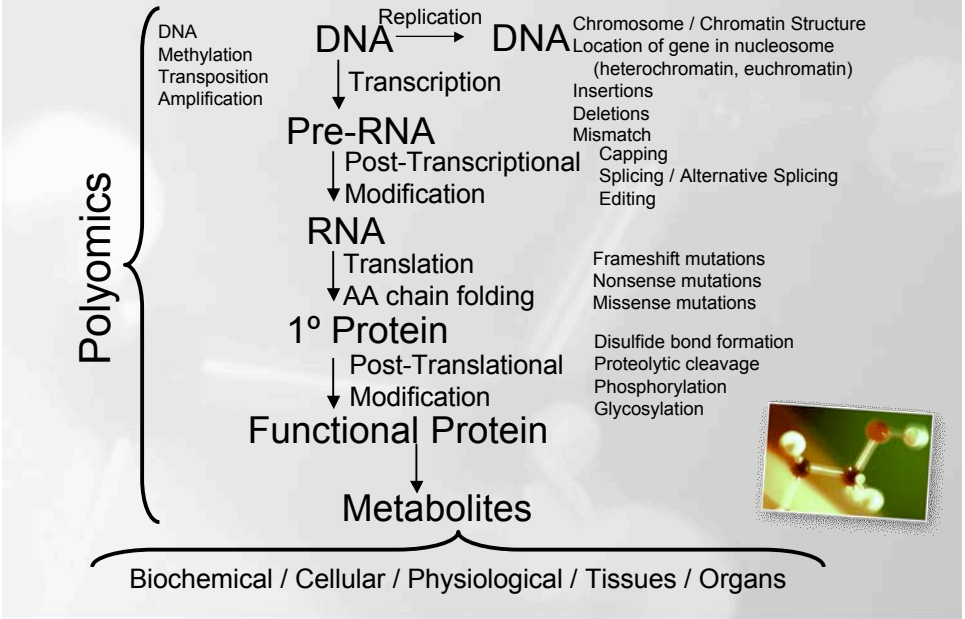
". . .the data integration method reported has highlighted a number of unexpected pathways that play important roles in modulating cellular toxicity after treatment with a damaging agent. . . .The approach is now ripe for screening . . .and has the potential to provide insight into the mechanism of action for many different compounds."
(emphasis added)

Sharan R *et al.* 2005. Conserved patterns of protein interaction in multiple species. Proc Natl Acad Sci U S A. 102(6):1974-9.

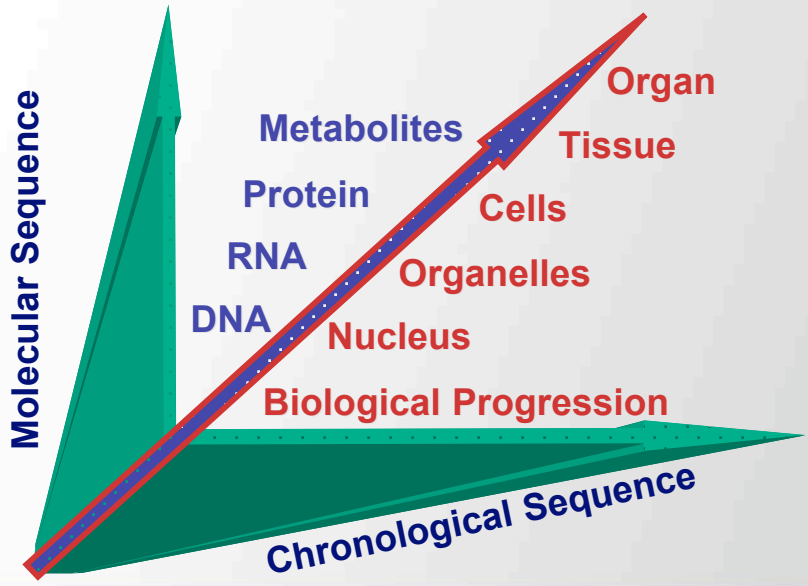
To elucidate cellular machinery on a global scale, we performed a multiple comparison of the recently available protein-protein interaction networks of Caenorhabditis elegans, Drosophila melanogaster, and Saccharomyces cerevisiae. This comparison integrated protein interaction and sequence information to reveal 71 network regions that were conserved across all three species and many exclusive to the metazoans. We used this conservation, and found statistically significant support for 4,645 previously undescribed protein functions and 2,609 previously undescribed protein interactions. We tested 60 interaction predictions for yeast by two-hybrid analysis, confirming approximately half of these. ***Significantly, many of the predicted functions and interactions would not have been identified from sequence similarity alone, demonstrating that network comparisons provide essential biological information beyond what is gleaned from the genome.***

Genomics to Risk Assessment

- Polyomic approaches provide molecular detail, but do not unequivocally identify the higher order physiological process affected by various genes, proteins, or metabolites, nor what changes at the molecular level will manifest detrimental physiological or organ-level changes.
- Ultimately, it will be necessary to understand the precise relationship between polyomics and these higher order functions in order to make meaningful interpretations of omic data for risk assessment.



Mechanisms Are Multidimensional



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Development of Western science is based on two great achievements: the invention of the formal logical system (in Euclidean geometry) by the Greek philosophers, and the discovery of the possibility to find out causal relationships by systematic experiment (during the Renaissance).

Albert Einstein to J.S. Switzer April 23, 1953. Albert Einstein Archives 61-381.

Progress Requires Tighter Thinking

"Toxicology is entering a new phase wherein powerful model systems will become available to predict toxicity and to study mechanisms of action. For these new techniques to achieve their potential, it will be necessary for toxicologists to pose precise questions, and to design experiments to answer those questions unequivocally."

John Ashby, 2000. Toxicol Lett 112-113: 3-8.

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Section 4: Progress

To remain relevant to 21st century science, toxicologists must face two challenges squarely. The first is to distinguish compensatory and adaptive responses from adverse effects; the second, to establish a clear conceptual model of causal relationships.

Christopher J. Borgert (today)

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