Polyomics: A Revolution in Mechanistic Risk Assessment?

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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

Fundamental Question

How do the new possibilities promised by omics technology meet the needs of risk assessment?

Example of Mis-Matched Thinking:

Toxicogenomics is the study of the structure and transcriptional output of the entire genome as it relates and responds to adverse xenobiotic exposure.

Risk Assessment is the process of defining adverse effects and estimating the probability of their occurrence in a population, including levels of exposure at which the probability is *de minimus*.

Solutions Require Tighter Thinking:

Ashby J. 2000. Toxicol Lett 112-113: 3-8.

"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."

Understanding Mode and Mechanism for Risk Assessment: Omic Profiling

Morgan et al. 2002. HERA 8(6): 1339-1353.

"With respect to risk assessment, it is important to distinguish "mechanism of action" from "mode of action." Transcript profiling can certainly aid in the latter, but the former is absolutely dependent on "one gene at a time" biochemical toxicology and molecular biology to determine the role of transcriptional responses in altering phenotype."

Definitions & Concepts

Borgert et al. 2004. Toxicol Appl Pharmacol 201: 85-96.

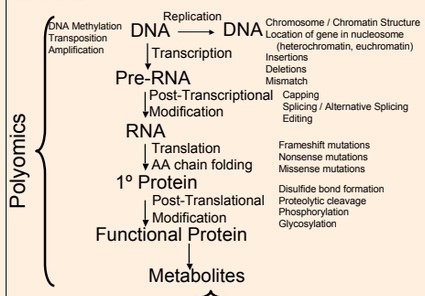
- **Mechanism of action:** a molecular sequence of events from absorption of an effective dose to production of a specific biological response.
- **Mode of action:** a set of physiological, biochemical and behavioral signs characterizing a specific biological effect.
- **Mechanism includes mode, *but not vice-versa*.**

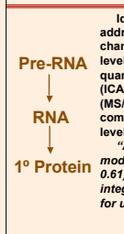
Mode of Action Classification Criteria

	EPA Cancer Guidance	EPA Cumulative Risk Grouping	EPA Mixtures Guidance	ATSDR Interaction Guidance	TEF Approach, Safe 1990, 1998
Molecular target	x	x	x	x	x
Cellular target	x		x		x
Physiological target	x				
Target organ	x	x	x	x	x
Toxic intermediates	x	x			x
Causality of steps	x				
Pharmacokinetics	x				x
Detox. pathways	x				x
Parallel DRCs	x	x			x

Various sets of criteria attempt to define the types and extent of mechanistic data required to determine the mode of action for a chemical or group of chemicals. There is no consensus about the requirements, other than to define molecular and organ level targets; omics addresses only molecular targets. (Borgert et al. 2004)

Polyomic approaches provide molecular detail (below), 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.



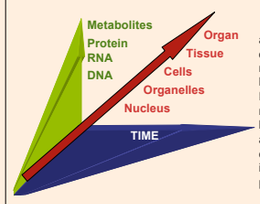


Ideker et al. (2001) is a landmark paper that directly addressed the question of whether the observed changes in mRNA expression were also reflected at the level of protein abundance. DNA microarrays, quantitative proteomics (using isotope-coded affinity tag (ICAT) reagents, and tandem mass spectrometry (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)." "These results underscore the importance of integrated mRNA- and protein-expression measurements for understanding biological systems."

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

Mechanisms Are Multidimensional



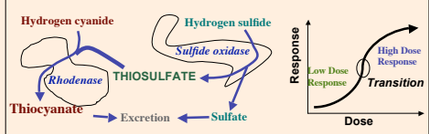
Mechanisms of chemical action are multidimensional not only in progression from the molecular to the organ level, but also in time. Furthermore, as effects at the molecular and cellular level lead to effects at the tissue and organ level, those changes can reciprocally impact cells and cellular processes.

Toxicity Metrics At Various Levels: What's the Dose?

	Dose	Biol. Effect	Interaction	Adverse Effect
Population	?			X
Individual	X			
Organ	?	X		
Cell	?	X		
Molecule	?		X	

Doses are often applied at one level of organization, e.g., whole animal, but measure effects, interpret interactions, and project adverse outcome at other levels of biological organization. Yet, we seldom have a reliable dose metric that inter-converts dose across levels. This *should* limit our interpretation of changes that occur at levels of biology where the dose is undetermined.

Dose-Dependent Transitions in Mechanisms



Above threshold, H2S & HCN inhibit mitochondrial cytochrome oxidase; same mode & clinical toxicity; assume additivity.

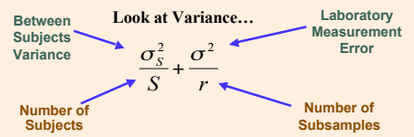
At subthreshold levels, effect would be antagonistic as thiosulfate from H2S metabolism increases HCN detoxification, reducing HCN toxicity.

Dose-dependent transitions could lead to different response curves in different dose ranges. Models must must have the flexibility to fit such responses.

(Borgert et al. 2004)

Allocation of Resources

•Understanding Variation is Key
•Careful Replication is Needed!



Two important components of variance are that due to subjects and that due to measurements. The subject variance is controlled by increasing the number of subjects, and the measurement error is controlled by increasing the number of replications per subject. If one is running microarrays, and the total number of arrays is fixed, then the balance between biological replications (subjects) and subsampling replication must be addressed.

Eight Mice Bio Rep

Control		Rχ	
1	2	5	6
3	4	7	8

Power = .56

Four Mice Chip Rep

Control		Rχ	
1	1	3	3
2	2	4	4

Power = .13

Sixteen Mice Pooled

Control		Rχ	
1,2	3,4	9,10	11,12
5,6	7,8	13,14	15,16

Power = .84

Resources Must Be Carefully Balanced

Suppose one is limited to 8 chips in a microarray experiment. Using one animal per chip (8 mice bio rep) provides a relatively low level of statistical power to detect a two-fold difference in gene expression(.56). Assuming consistency from chip to chip, reducing the number of animals and replicating chips (4 mice chip rep) actually decreases statistical power because the degrees of freedom are reduced from 6 to 2. Increasing the number animals and pooling samples from 2 animals per chip (16 mice pooled) provides an impressive increase in the statistical power of the experiment (.84). This underscores the need to verify consistency from chip to chip!

Experiment	Error df	Variance	Power
4 Mice Chip Rep	2	$\frac{\sigma_S^2}{2} + \frac{\sigma^2}{4}$	.13
8 Mice Bio Rep	6	$\frac{\sigma_S^2}{4} + \frac{\sigma^2}{4}$	.56
16 Mice Pooled	6	$\frac{\sigma_S^2}{8} + \frac{\sigma^2}{8}$	.84

Conclusions

1. **No single omic approach will define a mechanism - may need combination of polyomic and molecular biology approaches.**
2. **Understanding causal links requires several levels of biological organization beyond the molecular level (cellular, tissue, physiological).**
3. **Bioinformatics is essential for dose-response and temporal sequence analysis.**
4. **It is essential to differentiate between pre-toxicological changes and compensatory or protective responses to chemicals.**
5. **It is important to identify the dose-dependent transitions in mechanisms that most impact risk estimation.**
6. **Mechanistic and dose-response models must allow for dose-dependent transitions in mechanisms and in response parameters.**
7. **Polyomic approaches should plan for inference beyond omics:**
   a) **Omics measures only molecular resonses - e.g., gene expression levels and protein transcription;**
   b) **Need to chart pathways from gene interaction to toxicological endpoints that most impact risk estimation.**
8. **Relatively few polyomics studies have been completed to date that are sufficient to address risk assessment questions.**
9. **Obstacles include high cost of replicates, collection of data as dichotomous variables, bioinformatics, and biological relevance.**
10. **Uses of omics relevant to risk assessment include:**
    a) **As a marker of exposure**
       Selected Examples
       Mortuza et al. 2003
       Morgan et al. 2002
       Robertson et al. 2000
    b) **As a direct measure of toxicity**
       Selected Example
       Hamadeh et al. 2002
    c) **As a means of identifying or clarifying the mode or mechanism of toxicity for a chemical or class of chemicals**
       Selected Examples
       Sawada et al. 2004
       Kramer et al. 2004
    d) **Validated for risk assessment - None to date.**