Aquatic Systems & Environmental Health

Biomarkers

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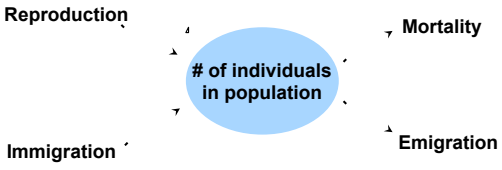
Challenge with ecotoxicology

- Important issue is population stability
- Identify causative agent(s) in a complex mixture of agents when inputs of the causative agent may be sporadic

Assessing chemical impacts on populations

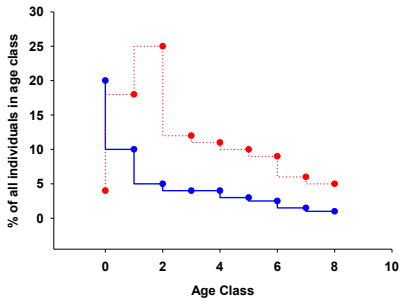
- What factors contribute to population levels?
- How would you determine which one is being affected in this case?
- If you believe it is an anthropogenic chemical that is impacting the population, how do chemicals get into water supplies?
- What happens to chemicals during this process?

Populations

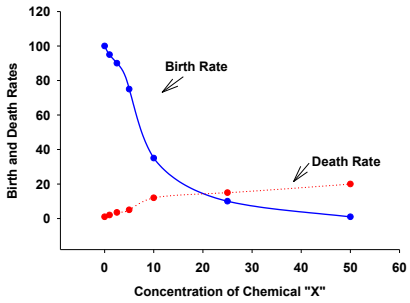


•How long an effect takes to manifest in population levels often depends on longevity and rate of reproduction

Population structure



Chemical Effects on Populations



Chemical Inputs into Water Sources

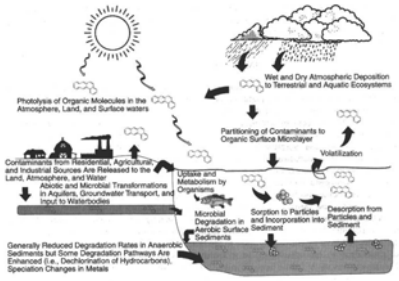


Figure 2.2 Some of the important physical and chemical processes that govern the fate and transport of environmental contaminants

From "Fundamentals of Ecotoxicology", Newman and Unger, eds.

What's happening to chemicals as they enter bodies of water?

- Dilution
- Microbial and photodegradation
  - Depends on chemical
- Binding to particulates and organic matter
  - Leads to sedimentation
  - Often related to hydrophobicity
- Bioaccumulation
  - May be none in the water, but very high in food items
  - Can lead to large differences in effect across species due to diet

What are the challenges for identifying impacts in aquatic toxicology?

- Systems are complex
  - Biological and chemical complexity
- Contaminant concentrations are often low
- Inputs are often sporadic
- In many aquatic systems, contaminants dissipate quickly due to flow
- This ain't CSI…
  - Analytical methods are incredibly sensitive, but you need to know what you are looking for

How can we narrow down the search?

•Look for changes in the organism that are indicative of exposure to specific chemicals or classes of chemicals

•Biomarkers or bioindicator

Quantifiable biochemical, histological or physiological measures that relate in a dose-or time-dependant manner the *degree of dysfunction* produced by contaminants (Mayer et al., 1992; in: Biomarkers, edited by Huggett et al., SETAC Press)

Types of Biomarkers

- Biomarker of Exposure
  - Measurement correlated with exposure of an organism to a xenobiotic substance
  - Not necessarily indicative of response
- Biomarker of Effect
  - Measurable biochemical, physiologic, behavioral changes in an organism that are recognized to lead to disease or health impairment

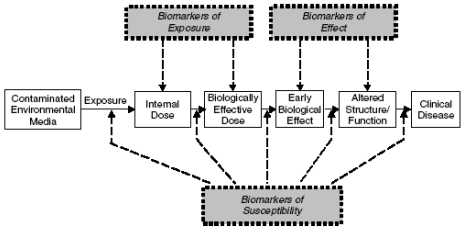


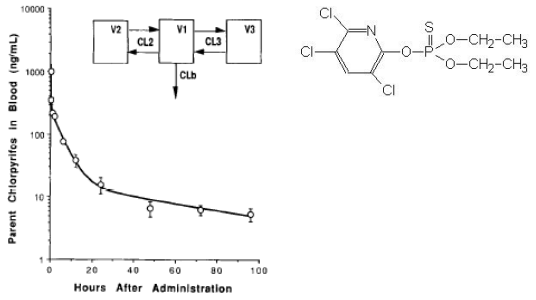
FIGURE 1. Exposure–disease continuum. The steps that are potentially monitored by biomarkers of exposure, effect, and susceptibility are indicated with dashed lines. Adapted from NRC (1987).

From Metcalf and Orloff, 2004

Biomarkers of Exposure

- Usually the exogenous chemical, its metabolites, or product of interaction between chemical and a target molecule.
  - Usually measured in easily obtained samples
  - May not identify source of exposure
- Long-lived chemical
  - Identify PCBs, dioxin, OCPs directly in blood or tissue
- Short-lived chemical
  - Identify metabolites

Elimination of Chlorpyrifos in catfish

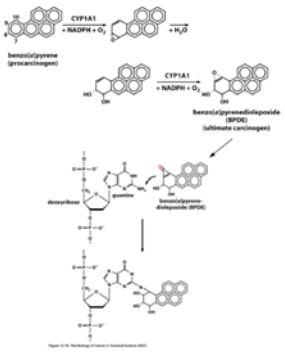


Barron et al., 1991, TAAP **108**:474

Adducts

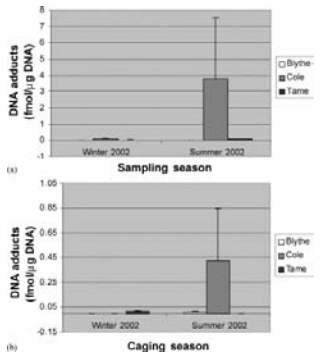
- When it is difficult to measure measure material directly, it is possible to look at reaction products of material
- Really only works for compounds that are reactive or have reactive metabolites
  - DNA
  - Protein
  - Indicative of reaction of active form of compound with biological material

BAP activation and adduct formation



Limitations of exposure biomarkers

- Provide information about absorbed dose, but don't tell you anything about whether or not the exposure caused an effect
- Detection can be misleading due to sensitivity of modern analytical instrumentation
- Association vs. Causation



From Winter et al., 2004, Mutation Res, v. 552

Concentration analogies

- **One-Part-Per-Billion**
  one 4-inch hamburger in a chain of hamburgers circling the earth at the equator 2.5 times
  one silver dollar in a roll of silver dollars stretching from Detroit to Salt Lake City
  one kernel of corn in a 45-foot high, 16-foot diameter silo
  one sheet in a roll of toilet paper stretching from New York to London
  one second of time in 32 years
- **One-Part-Per-Trillion**
  one square foot of floor tile on a kitchen floor the size of Indiana
  one drop of detergent in enough dishwater to fill a string of railroad tank cars ten miles long
  one square inch in 250 square miles
  one mile on a 2-month journey at the speed of light
- **One Part Per Quadrillion**
  one postage stamp on a letter the size of California and Oregon
  one human hair out of all the hair on all the heads of all the people in the world
  one mile on a journey of 170 light years

3. Biomarkers of Effects

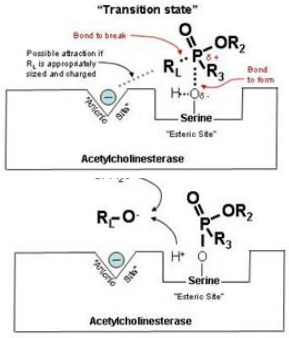
- Specific Biomarkers
- Broad Specificity Biomarkers
- Biomarkers under development

Specific Biomarkers

- Specific biomarker assays can stand alone and as such do not need chemical analysis or other biochemical tests for confirmation.
- Highly specific for individual chemicals
  - Inhibition of brain cholinesterase by organophosphate or carbamate insecticides

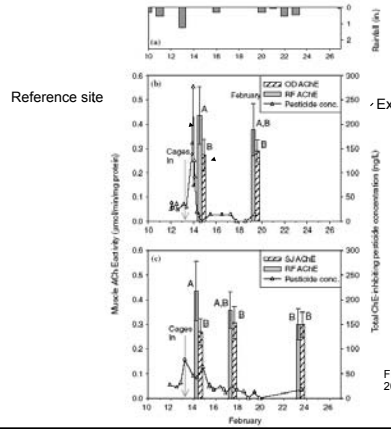
Cholinesterase Inhibition

•Organophosphate and carbamate insecticides bind to AChE and inhibit the enzyme
•Allows ACh to buildup, leading to overstimulation of receptors with ensuing SLUD symptoms



Cholinesterase Inhibition

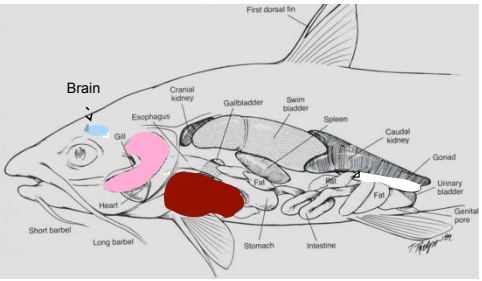
- Animals exposed to these chemicals will have decreased AChE activity
- Inhibition of brain AChE is well correlated with toxicity (though really need at least 50% inhibition to cause observable signs of toxicity).
- Activity usually remains depressed for days to weeks after OP exposure. This is good, because chemical itself is rapidly hydrolyzed in body and in environment.



Broad chemical response

- Cellular and organ level changes
- Biochemical changes
- Induction of vitellogenin in oviparous models
- Induction of metabolic enzymes
- Induction of metallothionein by metals

Cellular and Organ Responses



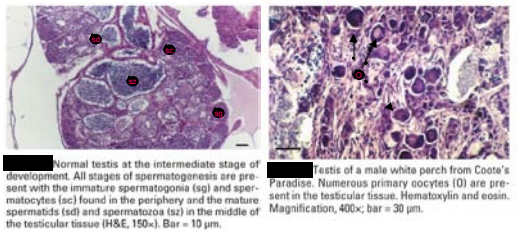
Brain, Gills, Liver and Gonads

Cellular Responses

- Cellular Morphology:
  - Histology & Histopathology
- Advantages:
  - Provide a way to look at the overall effect of exposures on cells and tissues
- Disadvantages:
  - Normal histology and variations sometimes poorly understood
  - Most evaluations are qualitative
  - Often can't discriminate causative agent

Cellular Responses

Feminization of male fish: Endocrine Disruption and Altered Gonadal Development in White Perch (*Morone americana*) from the Lower Great Lakes Region



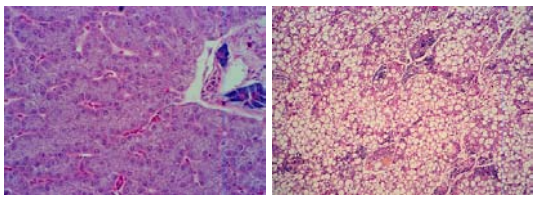
[redacted]Normal testis at the intermediate stage of development. All stages of spermatogenesis are present with the immature spermatogonia (sg) and spermatocytes (sc) found in the periphery and the mature spermatids (sd) and spermatozoa (sz) in the middle of the testicular tissue (H&E, 150×). Bar = 10 μm.

[redacted]Testis of a male white perch from Coote's Paradise. Numerous primary oocytes (O) are present in the testicular tissue. Hematoxylin and eosin. Magnification, 400×; bar = 30 μm.

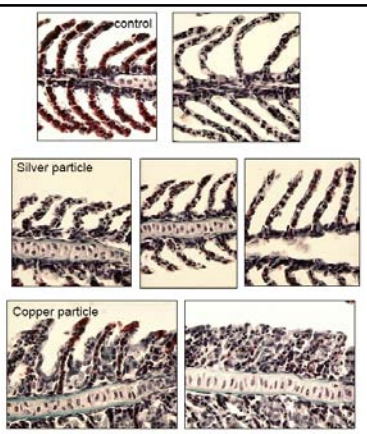
Source: Kavanagh et al., 2004

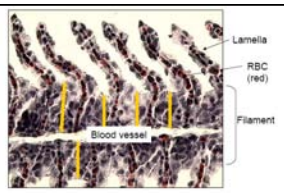
Cellular Responses

Largemouth Bass Liver

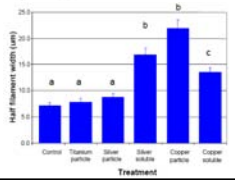


Normal Liver **Fatty Liver**





72 slides, 350 picture of filaments, 1750 measurements of half the width of a filament in micrometers (example yellow line)

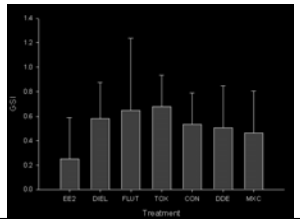


Organ Responses

- Organ Weights:
- Organosomatic Indices (%): (Organ weight/body weight) x 100
  - Liver: Hepatosomatic Index (HSI)
  - Gonads: Gonadosomatic Index (GSI)

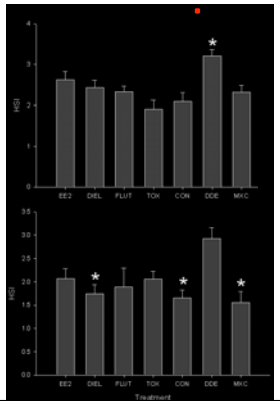
Gonadal Somatic Index

Female



Male

Hepatosomatic Index



Female

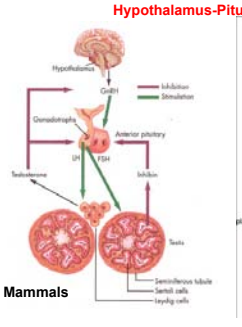
Male

Organ Responses

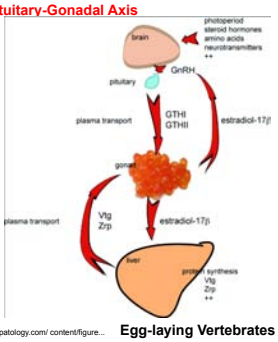
- Organosomatic Indices:
  - Varies greatly across species
  - Affected greatly by:
    - Nutritional state (liver)
    - Reproductive state (liver and gonads)
    - Exposure to contaminants: increase in HSI and a decrease in GSI
- Advantages: cheap & easy
- Disadvantages: non-specific

Biochemical Responses

Hypothalamus-Pituitary-Gonadal Axis



Mammals

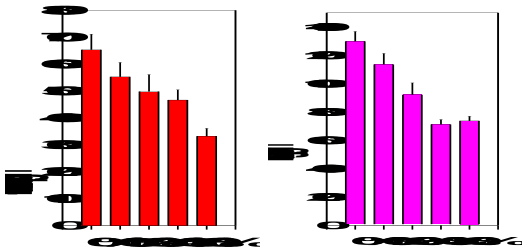


Egg-laying Vertebrates

Sources: www.homefertility.com/ hypothalamus.jpg and www.comparative-hepatology.com/ content/figure...

Biochemical Responses

Decreased sex steroid concentrations in plasma of largemouth bass exposed to paper mill effluents



Biochemical Responses

Decreased thyroid hormones in *Xenopus laevis* exposed to methoxychlor

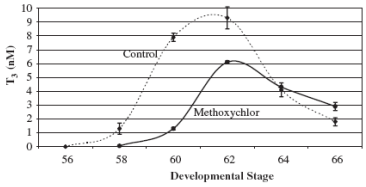
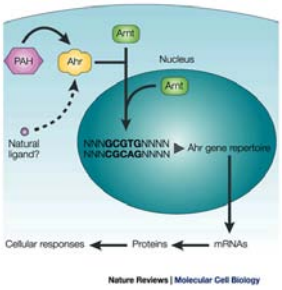


FIG. 4. Results of T_3 profile analysis of *X. laevis* control and 0.1 mg/l methoxychlor treatment specimens during the conclusion of prometamorphosis (stages 56–58) and throughout metamorphic climax.

Source: Fort et al. Toxicological Sciences 2004 81:454-466.

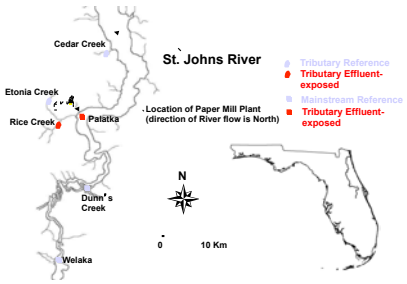
Metabolic Enzyme Induction

- Some P450s are inducible by exposure to xenobiotics
- CYP1A1 is strongly induced by compounds that bind to the arylhydrocarbon receptor (AhR) such as PAHs and dioxin
- Animals exposed to these chemicals will have higher levels of CYP1A1
- Induction can last for some time



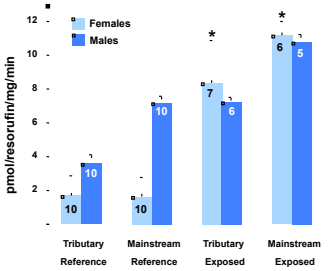
Effects of Paper Mill Effluents on Largemouth Bass Reproduction

Field Sites in North-East Florida



Proteins: CYP450s

Hepatic EROD activity as a measure of exposure to paper mill effluents



Source: Sepúlveda, Gallagher, Gross. (2004). Ecotoxicol. 13: 291-301.

Endocrine Disruption

- Many chemicals have the ability to alter function of the endocrine system
- May lead to reproductive or developmental problems
- A major group is chemicals that are considered "estrogenic" or interact with the estrogen receptor
- How can you determine if an organism has been exposed to an estrogenic substance?

Oocyte development and Vitellogenein

- major component of egg yolk.
- synthesized in the liver in response to estradiol and then secreted into blood for transport to developing follicles.
- Normally absent or very low in male and juvenile egg laying animals.

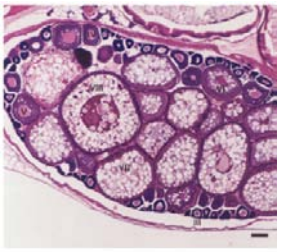
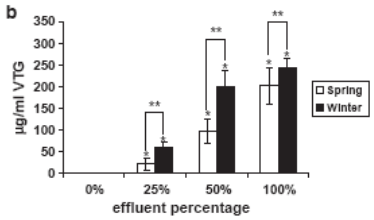


Figure 4. Normal ovary at late VtG stages of development. Pre-VtG oocytes (III and IV) are mainly located in the periphery, whereas VtG oocytes (V–VIII) are located in the middle of the ovarian tissue (H&E, 60×). Bar = 20 µm.

- Induction of Vtg is fairly sensitive marker of effect for estrogenic compounds in males and juveniles. Decreased Vtg in females has been correlated with poor reproductive success.
- Protein levels remain high in blood for weeks following exposure.



From Diniz et al., 2005, STE, v. 349

Metallothionein

- Small, thiol rich protein that strongly binds many divalent metals including cadmium, zinc, cobalt, copper and mercury
- MT is induced in response to these metals, so animals exposed to elevated levels of these metals will tend to have higher levels of MT.

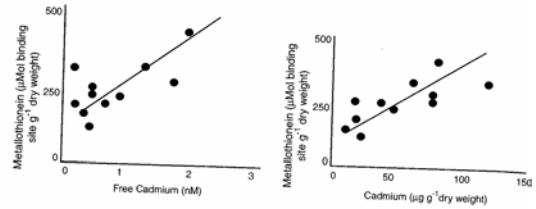


Figure 6.2 The relationships between free cadmium (Cd^{2+}) concentration in lake water (sediment-water interface) and metallothionein concentration in the freshwater mussel, *Anodonta grandis* (left panel) and cadmium concentration in the entire mussel and metallothionein concentration in this mussel (right panel). Each point represents samples from a different lake in the Rouyn-Noranda mining area of Quebec, Canada. (Modified from Figures 3 and 4 of Couillard, Y. et al., *Limnol. Oceanogr.* 38, 299–313, 1993.)

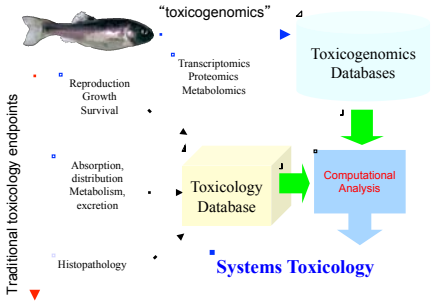
Identification of new biomarkers

- We are in an era of nearly exponential growth in our ability to measure things
- Huge interest in use of biomarkers to help replace traditional testing
- How do we identify new biomarkers?

What are "' Omics"?

- Large scale study of the transcriptome, proteome or metabolome
  - The entire transcriptional, protein or small chemical complement of a cell, tissue, or organism
- Particularly interested in elucidating the structure, function, and inter-relation of proteins
- Biology doesn't exist in a simple system. Since the 1990's, we have increased our ability to examine biological systems holistically. This is important for an accurate understanding of changes that occur in an organism as a result of disease or toxicity.

Toxicogenomics



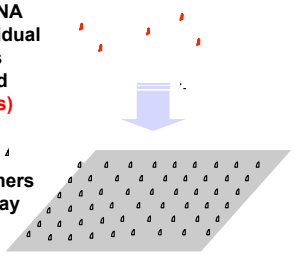
Types of "Omics"

Approach	Component examined	Techniques
Genomics	Genes	DNA sequencers
Transcriptomics	mRNA	Microarrays RNA Seq
Proteomics	Proteins	MS Protein chips
Metabolomics	Metabolites	GC-MS, LC-MS NMR

How arrays work.

Total RNA from individual fish is labeled (probes)

cDNAs or oligomers spotted on array (targets)



How arrays work.

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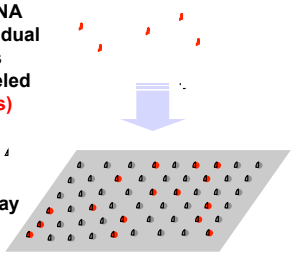
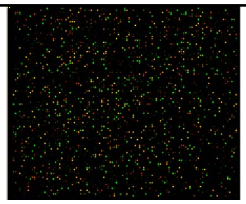


FHM Array

2,000 genes
Oligo-based array



Slide courtesy of EcoArray Inc.

FHM Array

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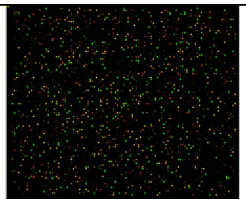
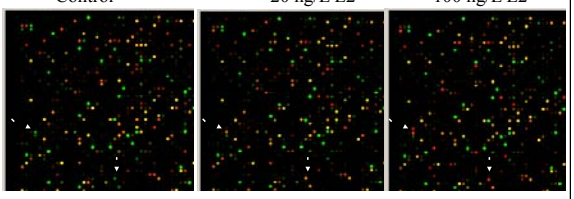


Control | 20 ng/L E2 | 100 ng/L E2



Slide courtesy of EcoArray Inc.

Why study the proteome?

- We already know many genomes and transcriptomes (and are rapidly sequencing more), so who cares about proteins?

Same Genome…..

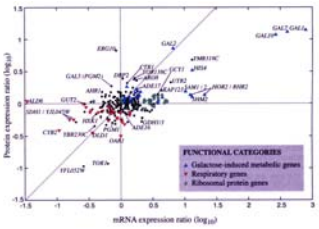
Different Proteome

Organisms have many transcriptomes and proteomes that vary with cell type, development, etc.

Why study the Proteome?

- Proteins are the effector molecules of a cell
  - Genes are the blueprint and mRNA is the CAD machine, but proteins are the actual parts
- The proteome is much more complex than the genome or the transcriptome
  - ~30,000 genes, 100,000+ transcripts, but 1,000,000+ unique proteins
- Really need to know all of the "omes" to understand system. Proteomics relies heavily on genomics for its success.

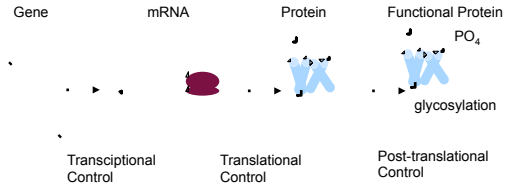
Does mRNA = protein?



•No!
•Analyses typically reveal less than 50% correlation of mRNA and protein levels

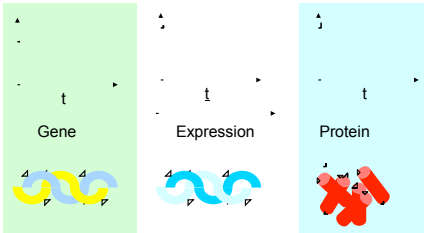
Idekar et al., Science 292:929 (2001)

Why does mRNA ≠ protein?

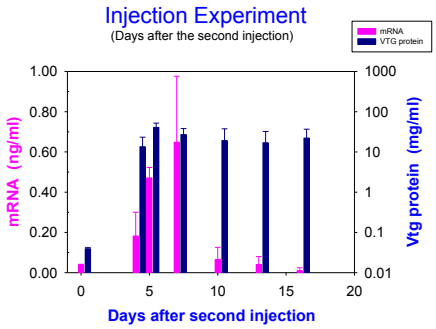


•Many genes can be alternatively spliced, yielding many mRNAs from a single gene
•Not all mRNAs are translated
•Most proteins are modified after translation

Temporal Changes in mRNA and protein

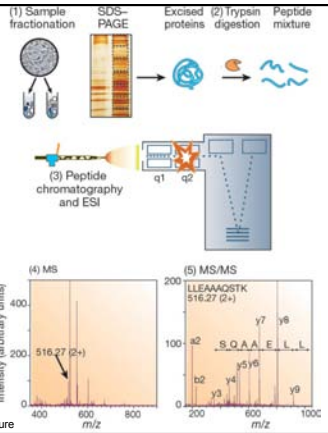


When you measure expression affects what you find



How is a typical proteomics experiment performed?

Pattersen and Aebersold, **2003**, *Nature Genetics*



Aebersold and Mann, 2003, Nature

How can proteomics be used?

- Understanding of cellular protein complexes and pathways
  - Protein profiling and organellar proteomics
- Understanding how disease or exposure alters cellular or tissue functions
- Identifying protein biomarkers
  - Disease diagnosis and prognosis
  - Tissue mapping
  - Identifying unknown exposures

Criteria for useful biomarkers

- Accuracy
- Reproducibility
- Sensitivity
- Specificity
- Plausibility
  - How good is the link with outcome
- Temporal characteristics
- Ease of sampling
- Throughput

Biomarker Interpretation

- Biomarkers are gaining increased importance in toxicology
- Caution must be used in extrapolation from one species to another
- Toxicokinetics is becoming more important than ever
- Experiments must be carefully designed and biomarkers appropriately validated

Concluding Thought

- When used properly, biomarkers are important tools for determining if organisms have been exposed to chemical pollutants and if organisms are being adversely affected by certain classes of chemicals.