NIEHS National Institute of Environmental Health Sciences

RISKeLearning

Computational Toxicology: New Approaches for the 21st Century

June 24th, 2009 Session 2: **Computational Toxicology: Dose Response Modeling**

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Srp Superfund Research Program

U.S. Department of Health and Human Services National Institute of Health National Institute of Environmental Health Sciences

Computational Systems Biology and Dose Response Modeling – Dioxins and Induction of Proteins in Liver

June 24, 2009

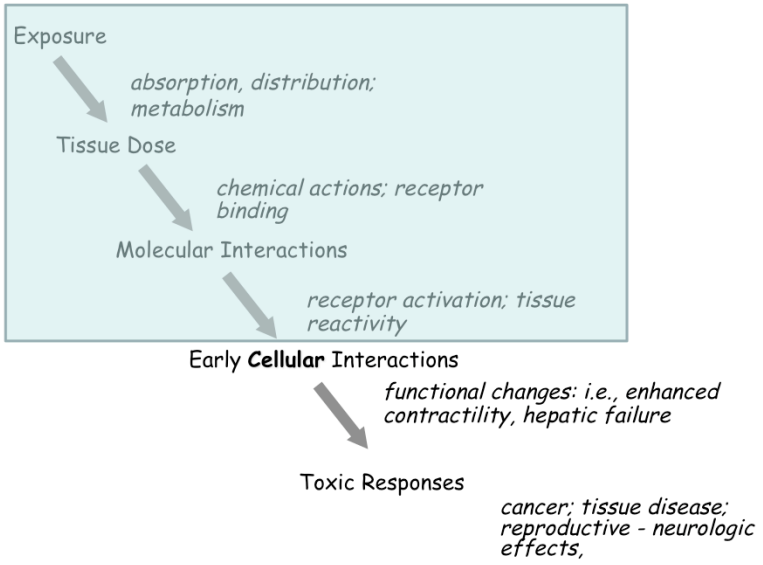
Spring/Summer 2009 edition of Risk e Learning
"Computational Toxicology: New Approaches for the 21st Century."

Melvin Andersen
Division of Computational Biology
The Hamner Institutes for Health Sciences

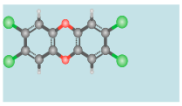
Computational Systems Biology and Dose Response Modeling – Dioxins and Induction of Proteins in Liver.

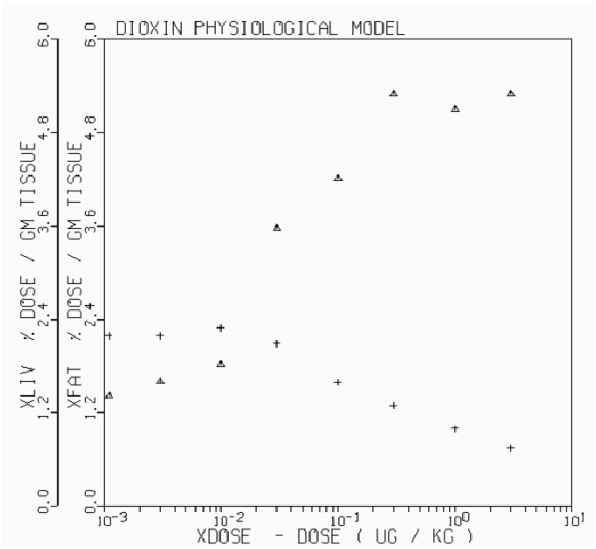
Regional induction of CYP proteins within the liver by dioxin indicated a switching between basal and fully-induced cells. Some switches in transcriptional states were known for positive feedback controlled synthesis of transcriptional factors (Andersen and Barton, Toxicol. Sci., 48, 38-50, 1999); however, computational tools were not well developed for assessing the networks and dose response characteristics for network activation by transcriptional activation. With support from Superfund Basic Research Project funds, scientists in the Computational Core at the Hamner Institutes for Health Sciences developed a course in Computational Systems Biology and Dose Response Modeling to provide instruction on using computational approaches in studying gene transcriptional processes in order to assess likely dose response behaviors for non-linear control processes inherent in biological systems (see The Hamner website: http://www.thehamner.org/education-and-training/current-course-offerings.html). This presentation provides background on dioxin induction of proteins in the liver and emphasizes the tools that can be applied in assessing the circuitry and dose response for these and other processes.

Exposure - Dose - Response Relationships

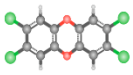


Dioxin distribution to Liver and Fat





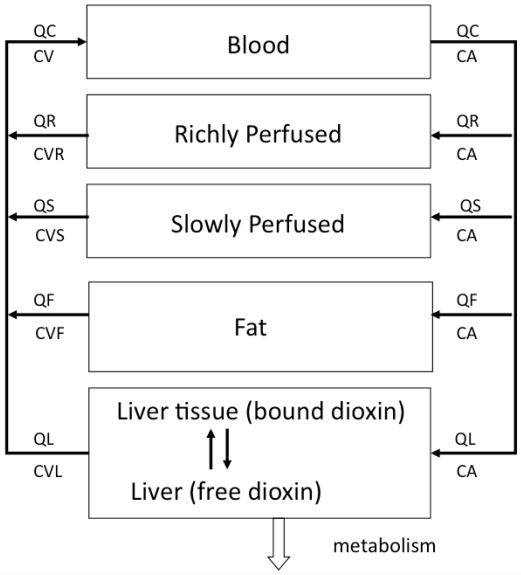
The dose-dependent curvature is due to induction of a dioxin binding protein in liver by treatment with dioxin.



Gene Induction in liver

- Dioxin caused increases in proteins in the liver that bind dioxin (CYP 1A2) and sequester the compound in liver
- How did we first account for increase in binding of dioxin in the liver with time?

PBPK Model Schematic



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Transcriptional Model (1988 & 1993)

Ah-TCDD

DRE ..... ⟺ m-RNA-CYP 1A2

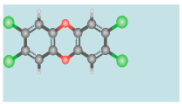
CYP 1A2 - Gene

$$Ah + TCDD \overset{Kb1}{\rightleftharpoons} Ah\text{-}TCDD$$

$$Ah\text{-}TCDD + DRE \overset{Kd}{\rightleftharpoons} Ah\text{-}TCDD\text{-}DRE$$

$$\text{DRE Occupancy} = \frac{Ah\text{-}TCDD}{Ah\text{-}TCDD + Kd}$$

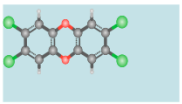
Kb1 – Ah receptor binding
Kd – Receptor complex with DNA

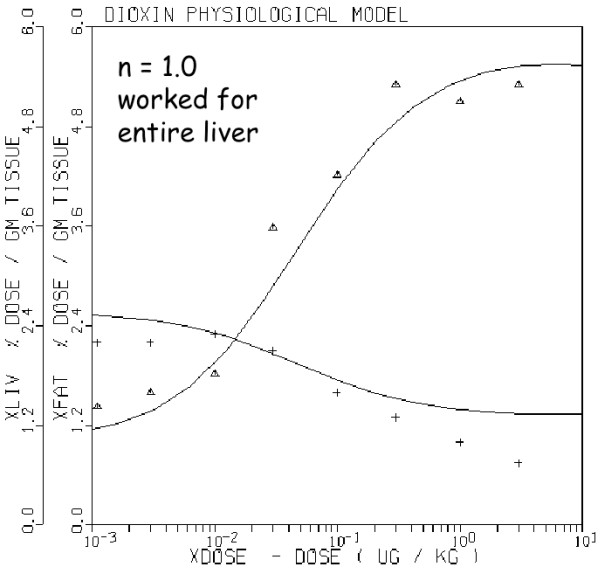


Model for Time-Dependent Protein Synthesis

Inducible synthesis (Ko; Kmax) → Binding protein & Enzymes → Degradation (Kelim)

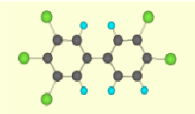
$$d(Pr)/dt = Ko + \frac{Kmax\,[Ah\text{-}TCDD]^{n}}{Kb1^{n} + [Ah\text{-}TCDD]^{n}} - kelim\,[Pr]$$

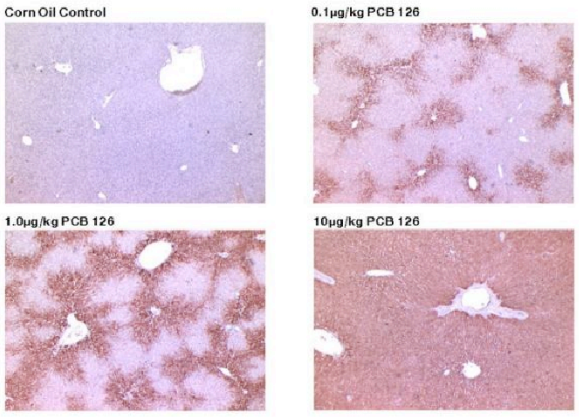




Need to fit to Kd, Kb1, Kb2,Bm2I, and n. Got some sense of the strength of binding, the maximal increase in protein from this approach. Is it reasonable?

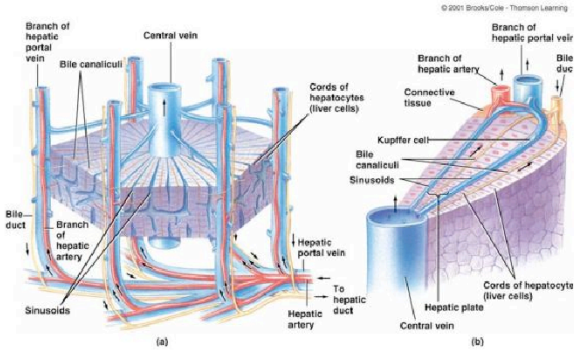
INDUCTION OCCURS IN SPECIFIC REGIONS OF THE LIVER ACINAR STRUCTURES – IT'S ABOUT CELLS





- The PBPK model for dioxin-induced protein expression needs to account for regional differences in response.

Liver Structure



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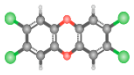
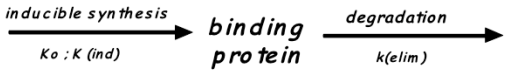
Creating a Multi-Compartment Liver Acinus:

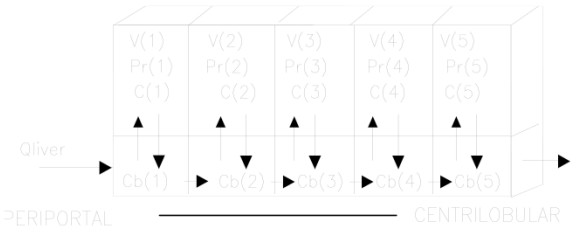


Induction Equations:



$$d[Pr]/dt = ko + \frac{k(max)\ [Ah\text{-}dioxin]^{n}}{Kb1^{n} + [Ah\text{-}dioxin]^{n}} - k(elim)\ [Pr]$$

Liver Bulk Structure:



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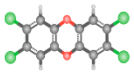
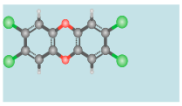
Visualization and Comparison with Immunohistochemistry



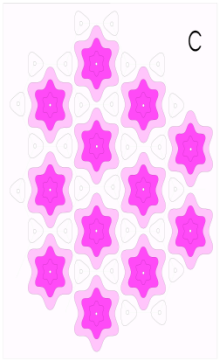
- Simulation of geometric organization is necessary. The predicted induction in the various sub-compartments was used to 'paint' regions in a two-dimensional acinus.

Representation of a field of acini in a liver section

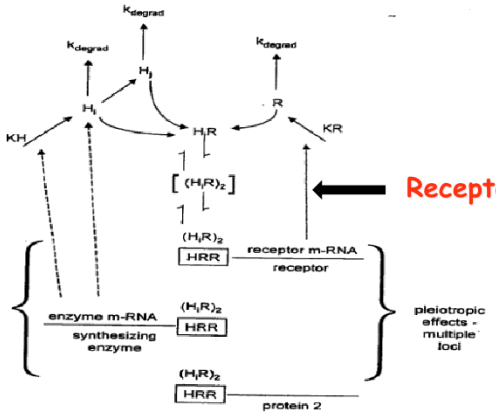


Modeling Regional Induction in Liver

- Requires high n-values
- Binding constants vary between adjacent zones
- **Very empirical**
- Nonetheless, induction is equivalent to a switch
- Need biological studies about non-linear switching



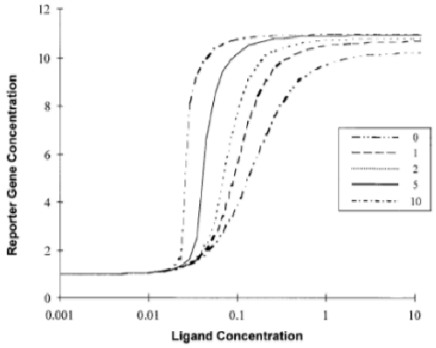
Making a Switch – Looking at Possibilities Positive Feedback



Andersen and Barton, Tox. Sci., 48,38, 1999.

Receptor Auto-Regulation Produces Steep Dose Response

- **Auto-induction** (positive feedback) of receptor protein by 2, 5, and 10-fold causes increasingly more steep dose-response curves.
- All-in-all we simply needed to do more than simulate sets of equations if we wanted to really understand the process.



Andersen and Barton, Tox. Sci., 48,38, 1999.

SBRP-How we got to the point of offering a course – Computational Systems Biology and Dose Response Modeling. Found a paper…..

SYSTEMS BIOLOGY: A USER'S GUIDE

PERSPECTIVES

ES

Back to the future: education for systems-level biologists

Ned Wingreen and David Botstein

Abstract | We describe a graduate course in quantitative biology that is based on original path-breaking papers in diverse areas of biology; each of these papers depends on quantitative reasoning and theory as well as experiment. Close reading and discussion of these papers allows students with backgrounds in physics, computational sciences or biology to learn essential ideas and to communicate in the languages of disciplines other than their own.

Nature Reviews Molecular and Cell Biology, November 2006

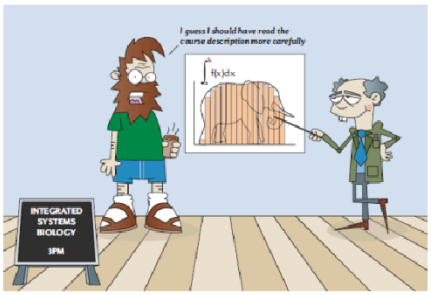


Figure 1 | Taking integrated systems biology a step too far.

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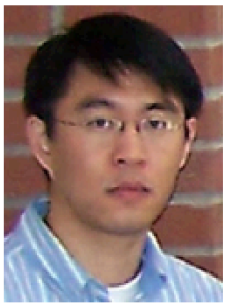
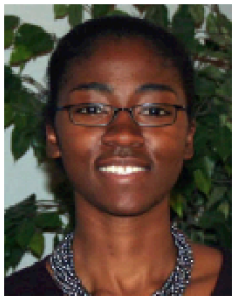
Dr. Qiang Zhang
Dr. Sudin Bhattacharya
Dr. Courtney Woods



Started a journal club inspired by Wingreen and Botstein paper.



Then, **somebody** thought it would be a good idea to do a course.

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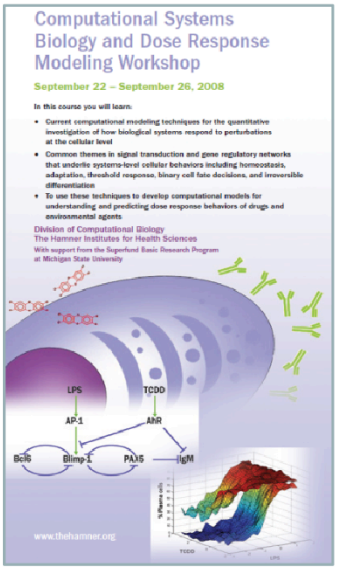

Full course with all lectures and exercises available at The Hamner website

You can find out the things I should have known in 1999

- Ultrasensitivity
- Feedback & Bistability
- Feedback Controllers and Loop gain
- Feedforward loops and transcriptional networks
- Non-linear dynamics versus solving equations

Some highlights follow:

http://www.thehamner.org/education-and-training/current-course-offerings.html

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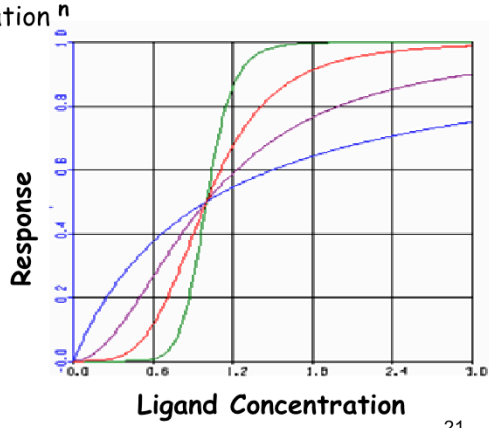
I. Ultrasensitivity

D-R Curves more steep than Michaelis-Menten:

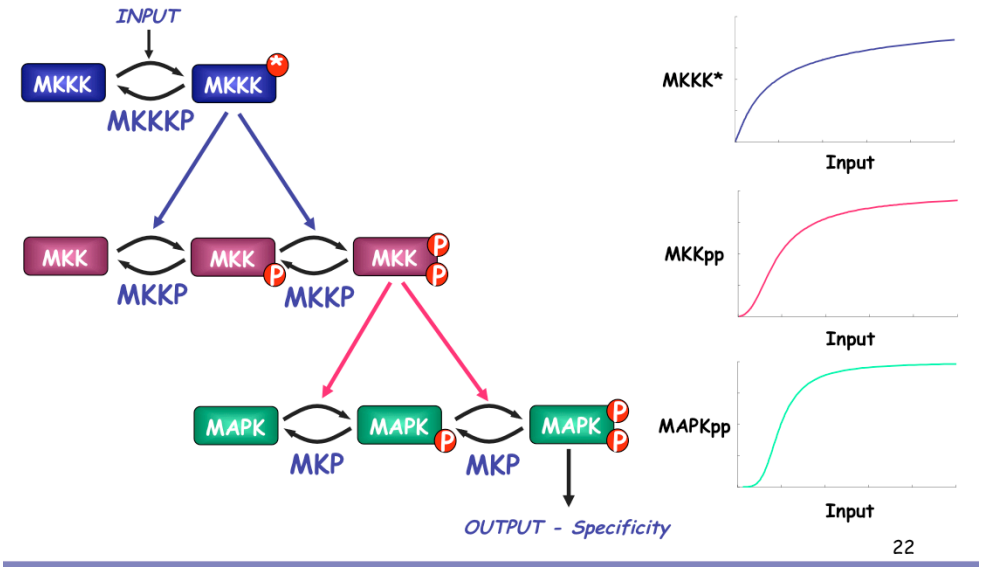
$$\text{Response} = \frac{\text{Rmax} \times \text{Concentration}^{n}}{\text{Kd}^{n} + \text{Concentration}^{n}}$$

N = 1, 2, 4, and 10

- What processes give rise to ultrasensitivity?
- Quite a few
- Very commonly – MAPK cascades

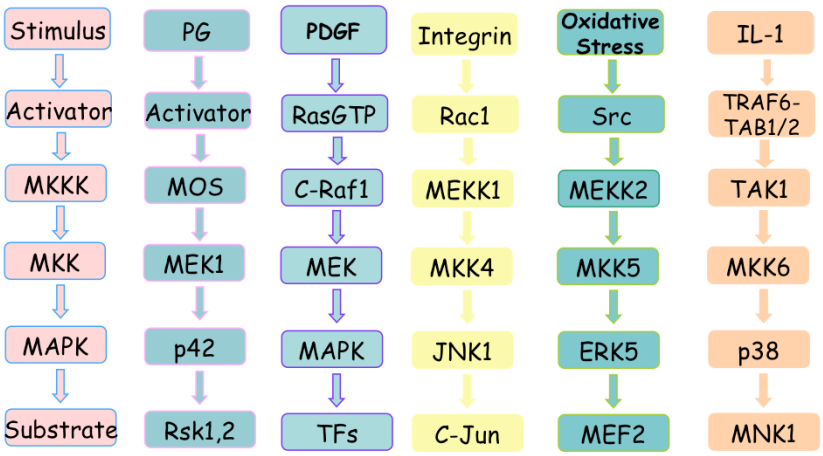


MAPK cascade is an ultrasensitive motif



We know MAPK appears in many signaling pathways. Then what's unique about MAPK, what does it do in terms of transferring signals. It turns out that MAPK cascade is a ultrasensitive motif.

And, a versatile signaling motif



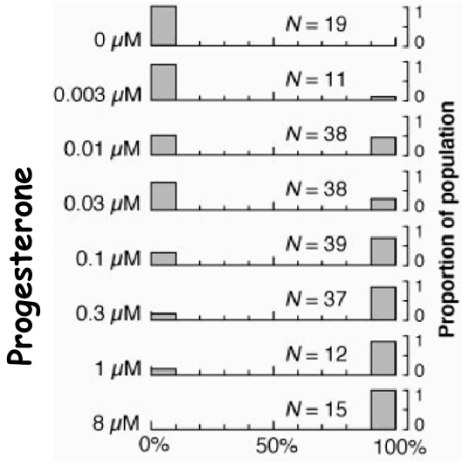
And others……. (Johnson and Lapadat, Science, 2002).

II. Bistability

- Delbrück (1948) proposed bistability as a general principle to explain discontinuous transitions in biochemical reactions
- Monod and Jacob (1961) proposed bistable gene regulatory circuits to explain cell differentiation
- Thomas (1978) showed a **positive feedback loop** to be a necessary element of a network for bistability and switching behavior

Huang, in *Computational Systems Biology*, Kriete and Eils, ed., 2005.

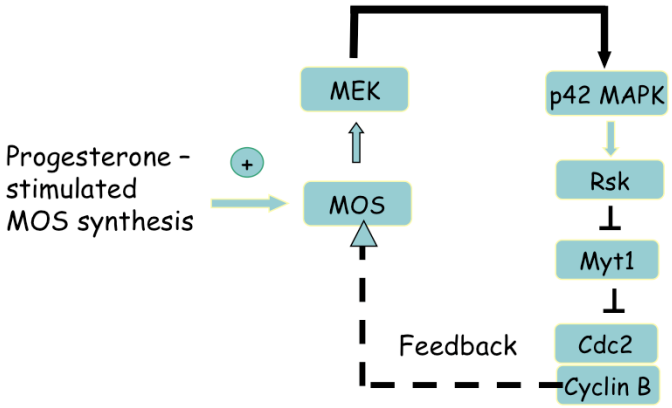
Moving from State-to-State



- Switch-like behavior occurs in oocyte maturation. Individual cells have either of two levels of MAPK-P: basal or fully induced. **(n ≃ 30)**

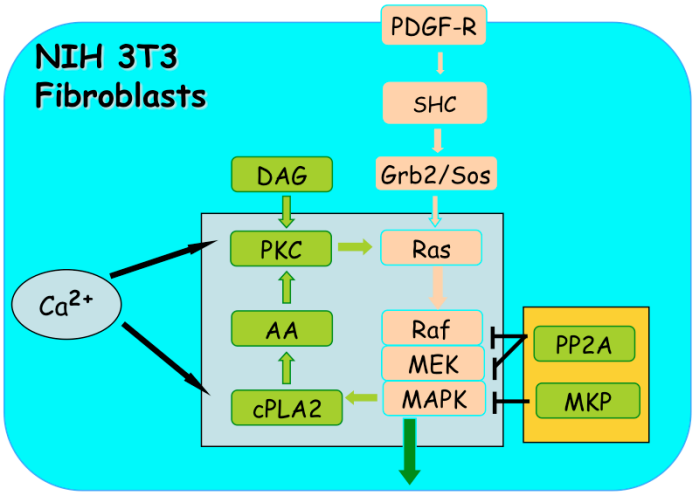
Ferrell and Machleder, Science, 280, 895, 1998.

With a positive feedback loop



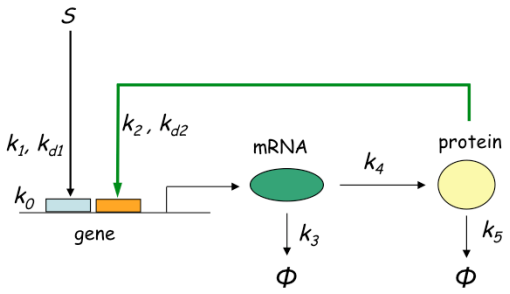
MAP-Kinase activation by progesterone acting via cell-surface receptors activating MOS.

Platelet Derived Growth Factor (PGDF)



- Positive feedback loop through cPLA2-AA-PKC

Ultrasensitive with feedback (Thomas, 1978)



$$\frac{dM}{dt} = k_0 + \frac{k_1 \cdot S}{k_{d1} + S} + \frac{k_2 \cdot P^n}{k_{d2}^n + P^n} - k_3 \cdot M$$

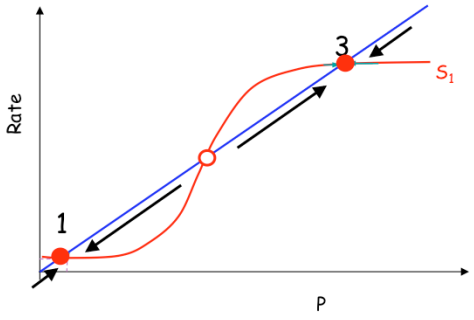
$$\frac{dP}{dt} = k_4 \cdot M - k_5 \cdot P$$

M: mRNA

P: protein

- A positive feedback loop

Bistability example: gene auto-regulation



$$\frac{dM}{dt} = k_0 + \frac{k_1 \cdot S}{k_{d1} + S} + \frac{k_2 \cdot P^n}{k_{d2}^n + P^n} - k_3 \cdot M$$

$$\frac{dP}{dt} = k_4 \cdot M - k_5 \cdot P$$

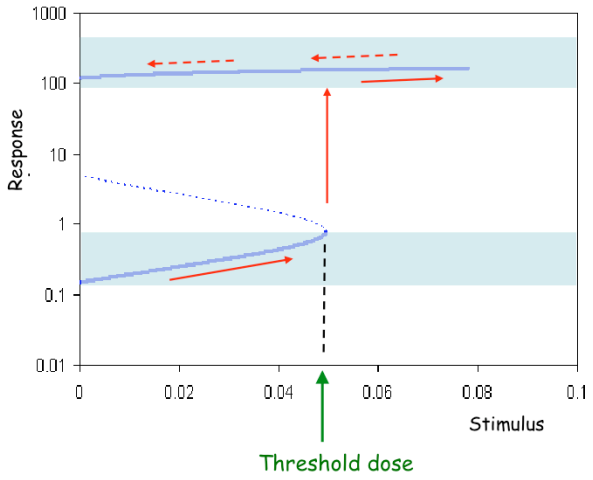
P synthesis rate

P degradation rate

1,2,3: *Steady states* (**synth rate** = **degrd rate**)

- 1,3: *Stable Steady states*
- 2: *Unstable Steady state*

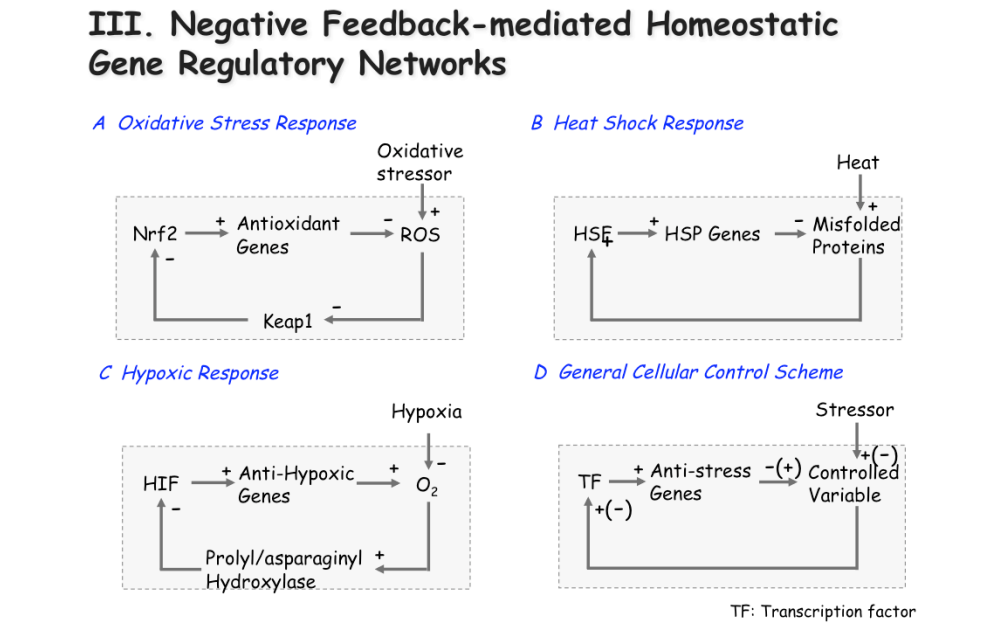
Bistability: "threshold dose" and hysteresis



Increasing dose

Decreasing dose

- Hysteresis:
  - biological "memory"
- Irreversibility
  - Stabilization of alternative states



cells *in vivo* must maintain a relatively stable intracellular micro-environment in an extracellular environment that is constantly changing and potentially unpredictable. Notably, many intracellular biomolecules need to be held within closely regulated ranges of concentrations for normal cell functions. Examples of these biochemical species, which could be detrimental and/or beneficial to cellular health, are reactive oxygen species (ROS), DNA adducts, misfolded proteins, O2, and glucose. When external stressors cause these molecules to deviate from their basal operating concentrations for extended period of time, normal cell functions become disrupted. As with many manmade control devices, such as thermostats and automobile cruise controls, homeostatic regulation of vital intracellular biochemical species appears to operate primarily via gene regulatory networks that are organized into negative feedback circuits.

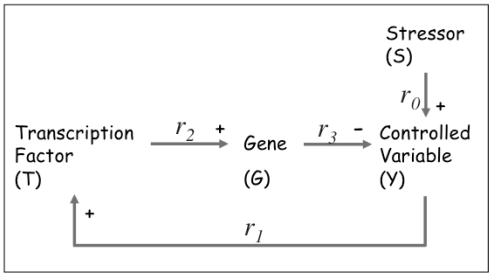
This is an example of oxidative stress response. Normal cell metabolism will produce ROS, which is eliminated by a set of anti-oxidants and enzymes. If the cell is under oxidative stress, ROS level increases initially. The increased ROS inhibits a protein called keap1, which negatively regulates Nrf2. This results in activation of Nrf2, which in turn upregulates a suite of anti-oxidant genes that accelerate ROS elimination, just bringing ROS back close to the normal level.

For heat shock responses, high temperature cause an increase in the amount of misfolded proteins. This increase in misfolded protein will titrate HSP away from HSF, thus indirectly activate HSF. More HSF upregulates HSP proteins expression, which functions to refold misfolded protein back to normally folded state.

A third example is the hypoxic response. If for some reason, the intracellular O2 level drops, hydroxylase will sense the situation, which in turn disinhibit HIF activity. High HIF levels drive up anti-hypoxic genes that functions to increase the supply of O2 to the tissue and cells.

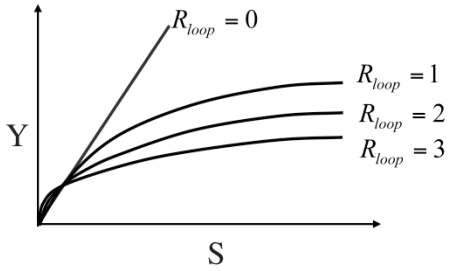
Together With other unlisted examples, we can generalize most of anti-stress gene regulatory networks into a common control scheme. The output of the system, referred to as controlled variable, is the biochemical species that is subject to perturbation by external stressors and therefore needs to be tightly controlled. The system contains specific transcription factors that serve as transducers to either directly or indirectly sense the level of the controlled variable. (In this fashion, alterations in the concentration of the controlled variable affect the activity or abundance of the transcription factor). Activated transcription factors then upregulate expression of individual or suites of anti-stress genes, many of which encode enzymes that participate in an array of interconnected biochemical reactions to counteract the perturbation to the controlled variable.

Y vs. S Dose Response with Constant Local Gains



$$R_S^Y = \frac{d\ln Y}{d\ln S} = \frac{1}{1+|r_1 r_2 r_3|} <= 1$$

$R_{loop} = |r_1 r_2 r_3|$



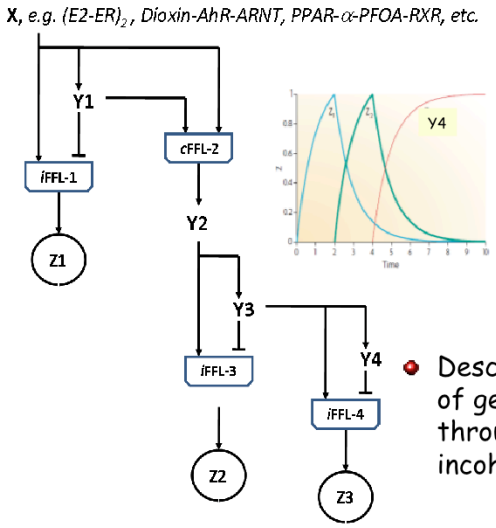
- The higher the R_{loop}, the more superlinear the Y vs. S curve.
- For high homeostatic performance, high R_{loop} is preferred !

* Assume all local gains remain constant with respect to S

Now let's focus on Y. According to the equation, for the controlled variable Y, RYS is always less than or at best equal to 1 since the loop gain >=0 (zero is equivalent to open loop). Therefore the Y vs. S dose response curve is superlinear or at best linear. The larger the loop gain, the smaller RYS is, the more superlinear the dose response curve becomes, and Y is more insensitive to changes in S. Since the goal of the feedback gene regulation is to maintain homeostasis for Y (which could be ROS, DNA adduct, misfolded protein, etc.), it is desirable to have the loop gain as large, hence RYS as small, as possible, in order to effectively resist perturbations by stressors.

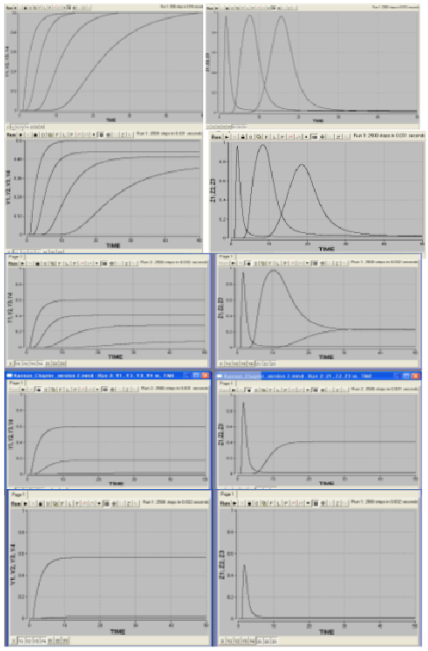
Augmentation of loop gain can be achieved by increasing local gain, either alone or in combination. Cells are furnished with many biochemical reactions/interactions or functional modules that can transfer signals in an ultrasensitive, or even switch-like manner, and thereby enhance local gains.

IV. Transcriptional networks



- Describing a cascading series of gene expression levels through coherent and incoherent feedforward loops

Alon, U. (2007). Nature Reviews Genetics, 8, 450-461.



Dose affect levels differentially

2.0 units

0.8 units

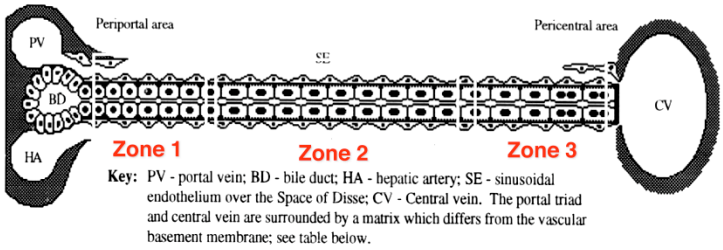
0.6 units

0.4 units

0.2 units

34

V. Gene dosage in liver cells



Key: PV - portal vein; BD - bile duct; HA - hepatic artery; SE - sinusoidal endothelium over the Space of Disse; CV - Central vein. The portal triad and central vein are surrounded by a matrix which differs from the vascular basement membrane; see table below.

Zones		1	2	3
Ploidy	rats	2N	4N4N & 8N	
	mice	2N & 4N	4N & 8N	up to 32N
	humans	2N	2N2N & 4N	
Growth		maximum	intermediate	negligible

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Mel Andersen - eRisk Learning Computational Toxicology; New Approaches for the 21st Century June 24, 2009

What might be going on with dioxin in liver, using computational systems biology lingo.

- The response to dioxin is activation of a **negative feedback loop** to maintain 'homeostasis' for a bio-active ligand-receptor complex (see Nebert, 1994).
- High loop gain is partially achieved by **ultrasensitivity** in the activation of the phase I enzymes – likely through MAPK pathways and **feedback-linked bistability**
- The variability in induction across the liver may be due to the 'differentiation' state of the hepatocytes along the sinusoid, partially determined by ploidy state and **gene dosage**
- The **transcriptional network** activated is likely to differ across the sinusoid and in the periportal area to include proliferative responses and hyperplasia.

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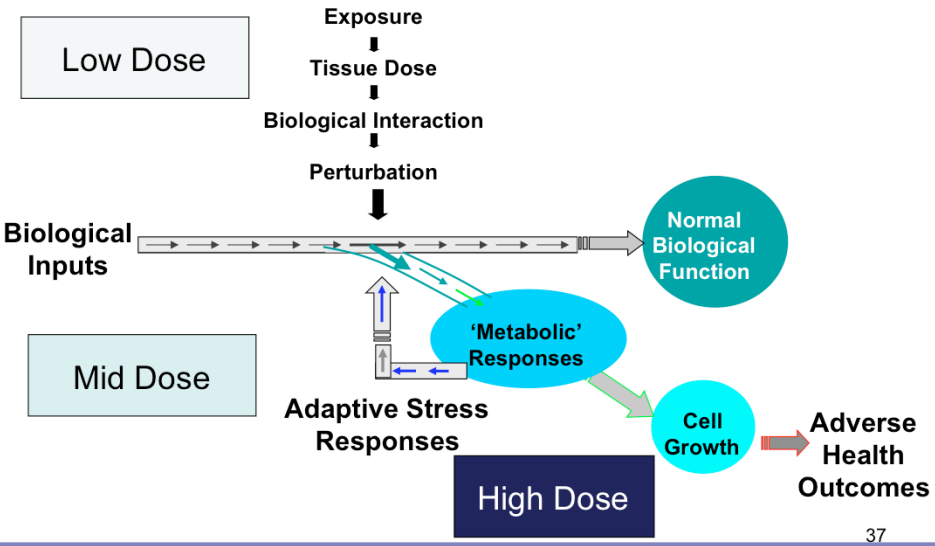
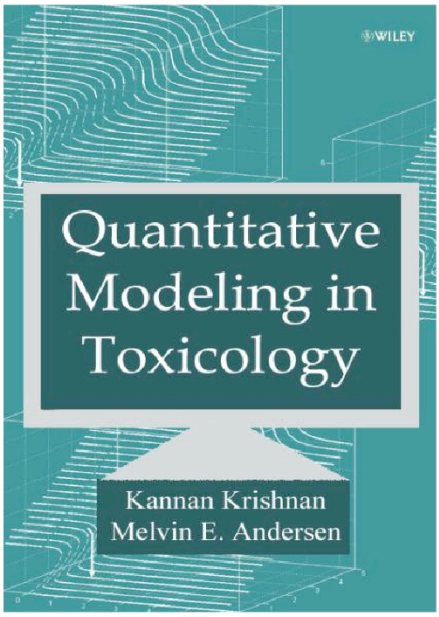
Dose Dependent Transitions – Hypothesis



37



For more information -
scheduled publication 12/2009

Some chapters
- dioxin example
- ultrasensitivity
- bistability
- transcriptional networks

Include exercises from the Hamner/SRP Course; other chapters cover PBPK modeling

Thanks again to:

SRP funding
Qiang Zhang
Sudin Bhattacharya
Courtney Woods

38

Studying the Basic Biology of B cell Differentiation to Understand the Effects of 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD) on Immune Function

Spring/Summer 2009 edition of Risk e Learning
"Computational Toxicology: New Approaches for the 21st Century."

Rory B. Conolly
National Center for Computational Toxicology
Office of Research and Development
USEPA

Norbert E. Kaminski
Center for Integrative Toxicology and
Department of Pharmacology & Toxicology
Michigan State University

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Disclaimer

Although this work was reviewed by EPA and approved for publication, it may not necessarily reflect official Agency policy.

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B cell biology

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Profile of Biological Activity by TCDD

- enzyme induction
- hepatomegaly
- lymphoid involution:primarily thymus
- immunomodulation (i.e., mostly suppression)
- chloracne and epithelial hyperplasia
- teratogenesis (example: cleft palate)
- cancer (tumor promoter)
- wasting syndrome
- death

42

Why focus on the B cell?

- The antibody response has historically been one of the most sensitive indicators of TCDD immunotoxicity

- The magnitude of humoral immune suppression by TCDD is similar for antigens requiring different cellular cooperativity (sRBC, DNP-Ficoll) and to the polyclonal B-cell activator LPS

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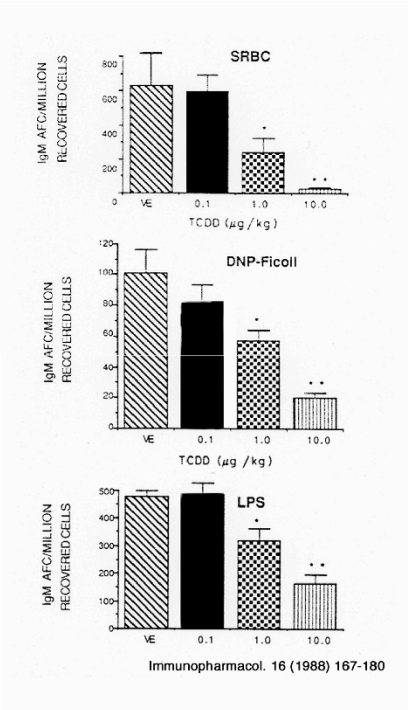

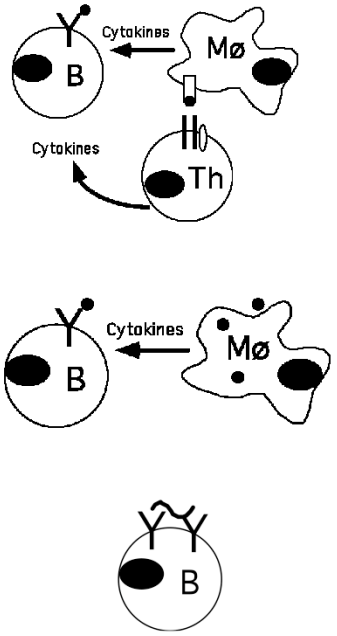

Dooley and Holsapple Immunopharmacology (1988)

Why focus on the B cell?

- Spleen cell separation-reconstitution experiments show that the B-cell is the primary cellular target within leukocyte subpopulations

- Direct effects of TCDD on B cell function have been demonstrated in purified primary B cells and B cell lines.

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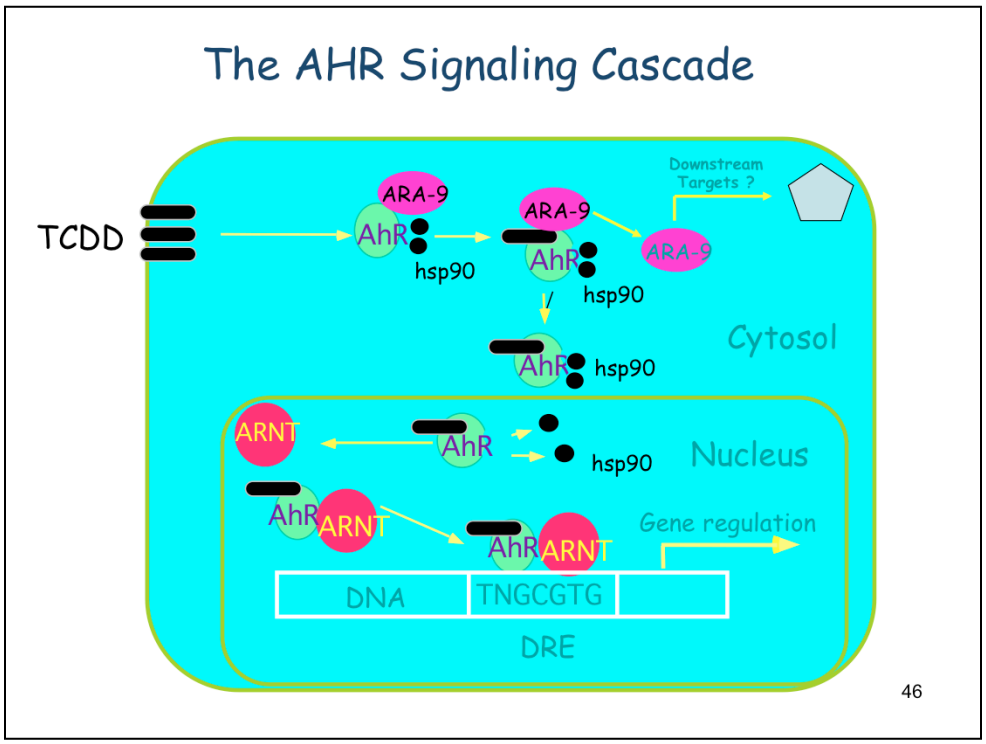
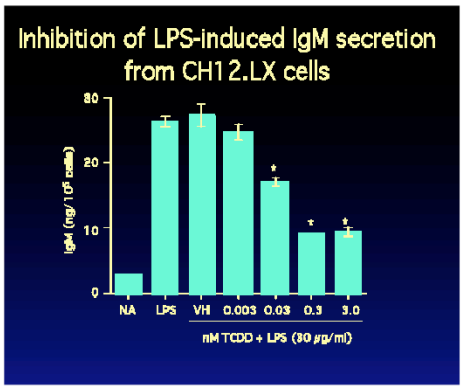
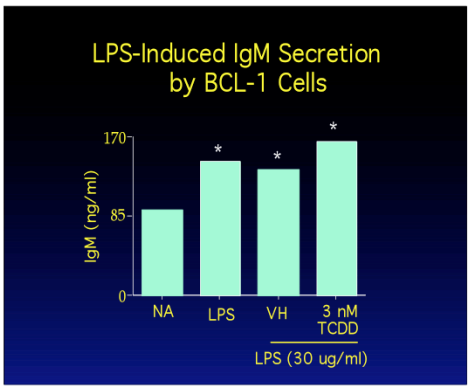

The AHR Signaling Cascade
TCDD
ARA-9
AhR
hsp90
ARA-9
AhR
hsp90
Downstream Targets ?
ARA-9
Cytosol
AhR
hsp90
ARNT
AhR
hsp90
Nucleus
AhR
ARNT
AhR
ARNT
Gene regulation
DNA
TNGCGTG
DRE
46

Role of the AHR in the Suppression of the IgM Response by B Cells

AHR+

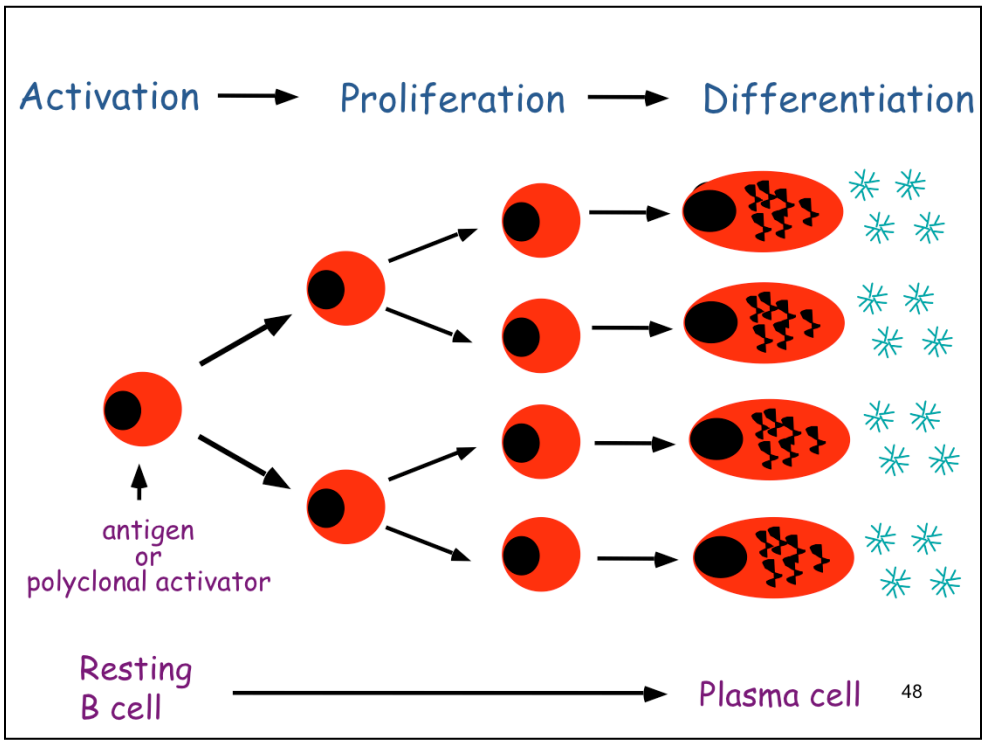


AHR-

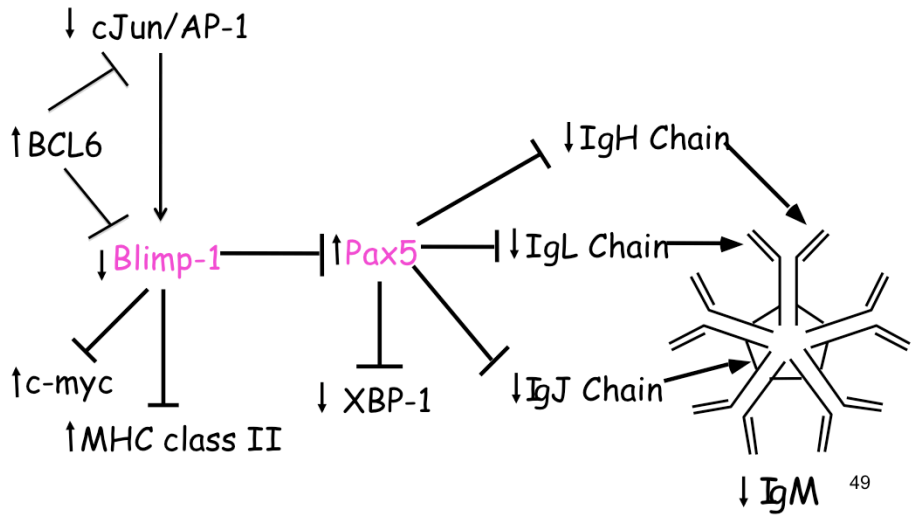


47

Sulentic et al., Mol Pharm, 53:623-629 (1998)


Activation
Proliferation
Differentiation
antigen
or
polyclonal activator
Resting
B cell
Plasma cell
48

Mediators of the B Cell Differentiation Program (Resting B cell)



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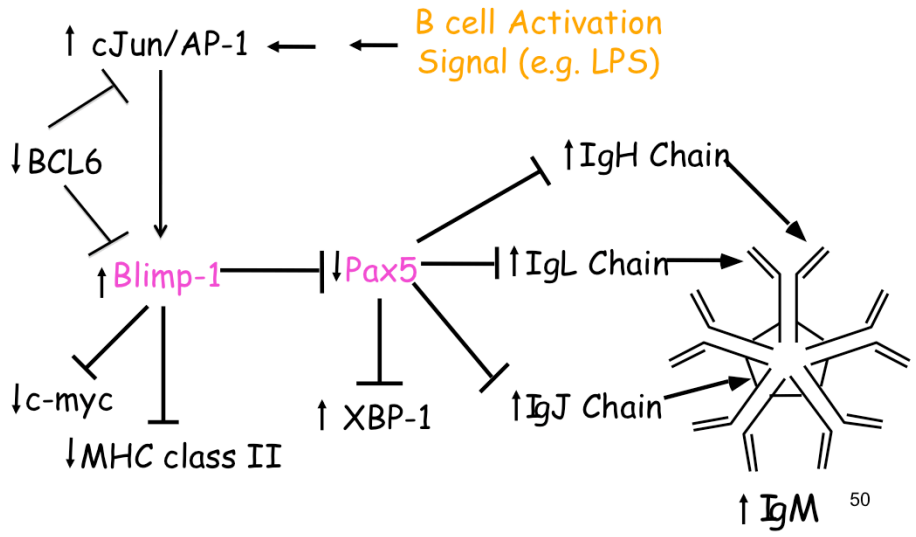
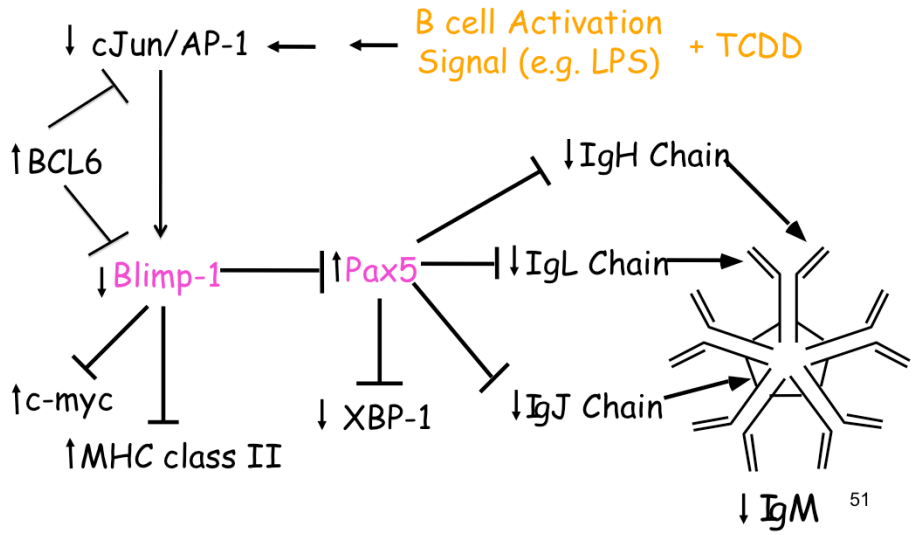
Mediators of the B Cell Differentiation Program (Plasma Cell)



Mediators of the B Cell Differentiation Program with TCDD (Plasma cell?)



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Summary

Suppression of the IgM response by TCDD is:

- mediated through direct effects on the B cell
- dependent on the AHR
- due, in part, to impaired B cell differentiation

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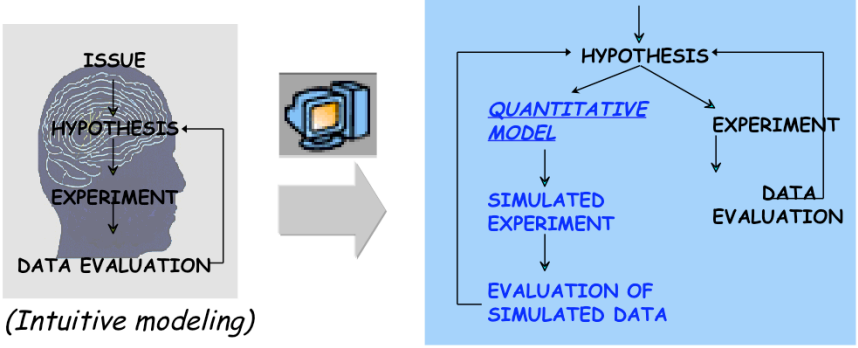
Computational modeling

53



(Intuitive modeling)

(Formal + intuitive modeling)

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Learning from models

- *All models are wrong but some are useful.*

George Box

55

Learning from models

- *All models are wrong but some are useful.*
- Ask, not if the model is right, but can we learn something useful from it?

George Box

56

Philosophy

- Develop the model to help us better understand what the data can tell us.
- Model is interpretive and predictive.
- Using good practice, more likely to uncover uncertainty that introduce it.
- Not required to be “right”.
- Is required to be better than no model!

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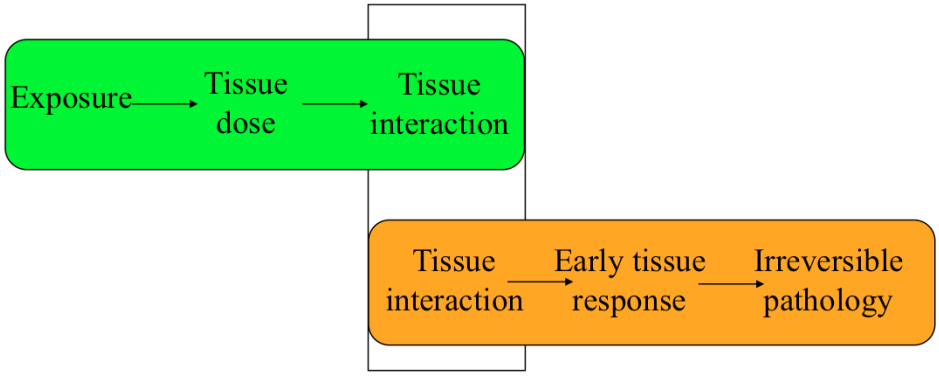
Exposure-response continuum



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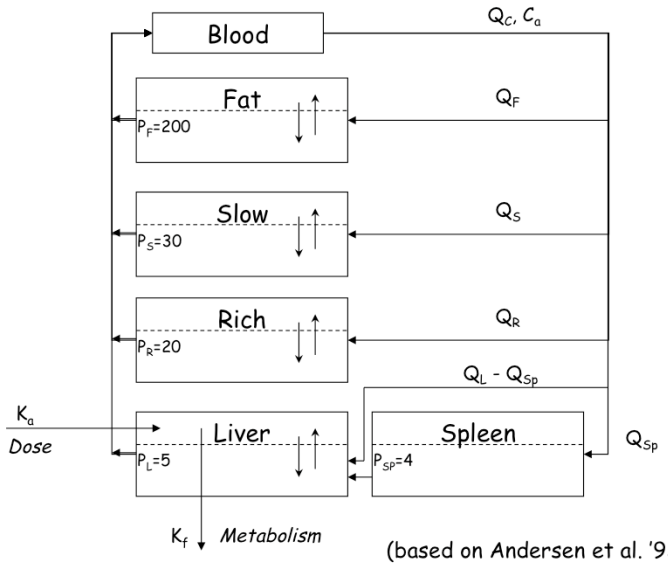
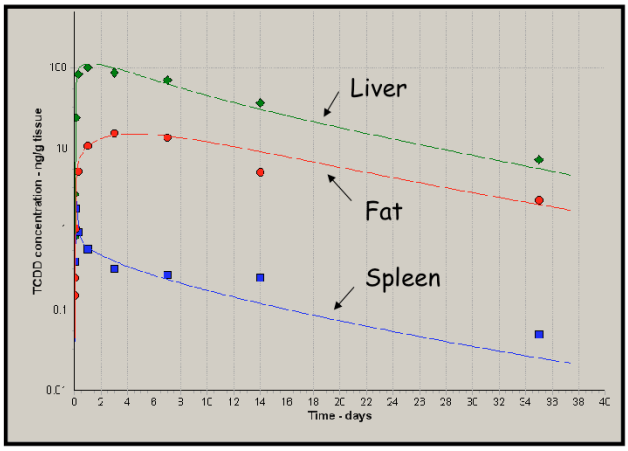
Dioxin PBPK Model with Spleen



(based on Andersen et al. '93, Wang et al. '97)

Dioxin PBPK Model with Spleen - Fitting long time-course rat data

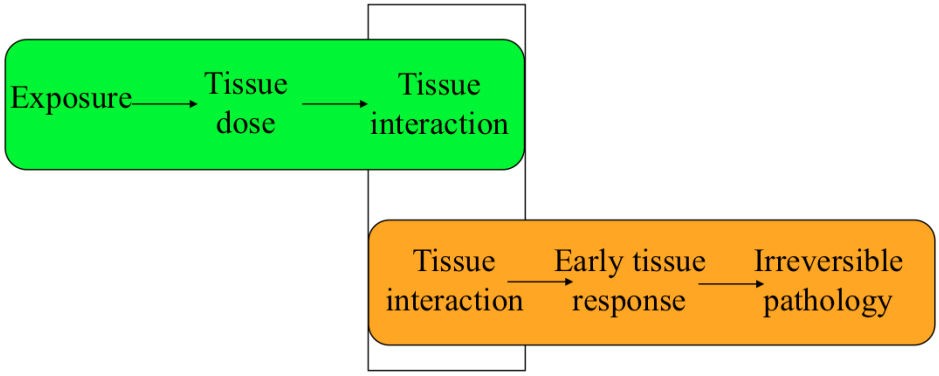


Oral dose: 10 µg/kg

60

(Wang et al. '97)

Exposure-response continuum



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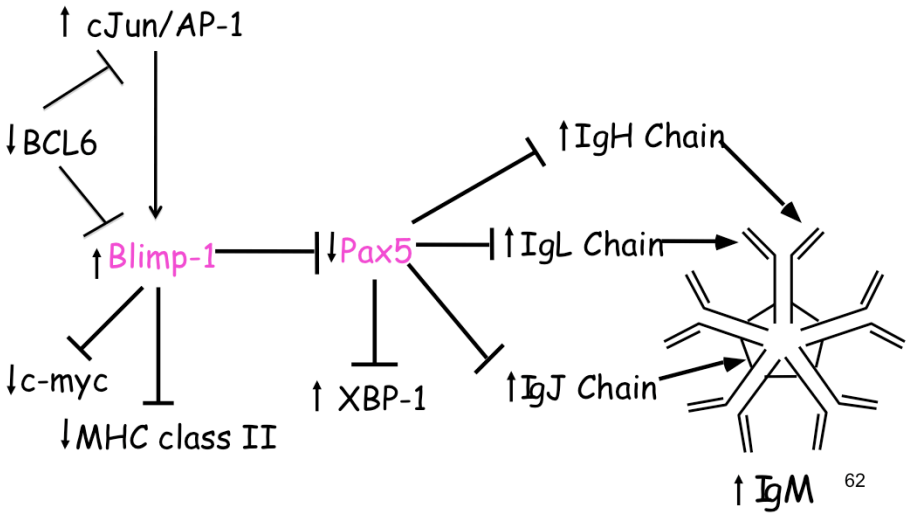
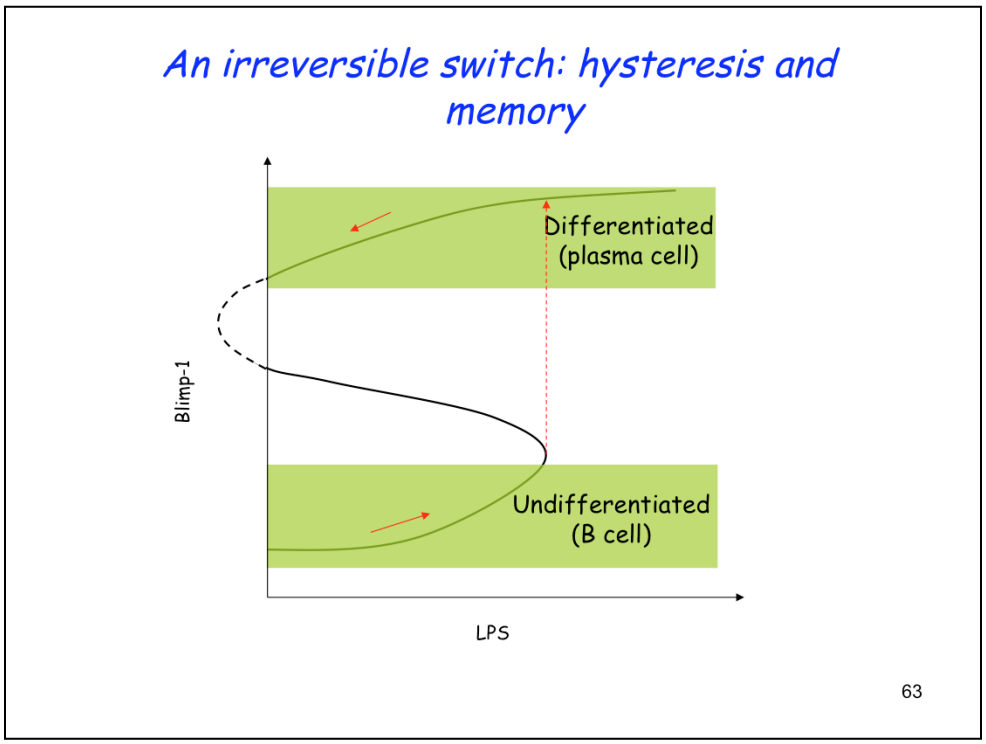
Mediators of the B Cell Differentiation Program (Plasma Cell)



62

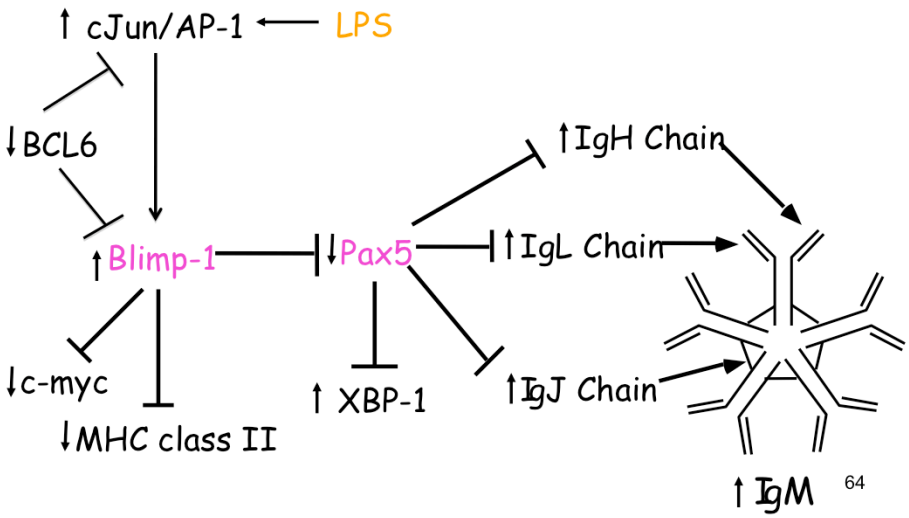


More lines of evidence that there may be more interactions working to make a robust irreversible switches.

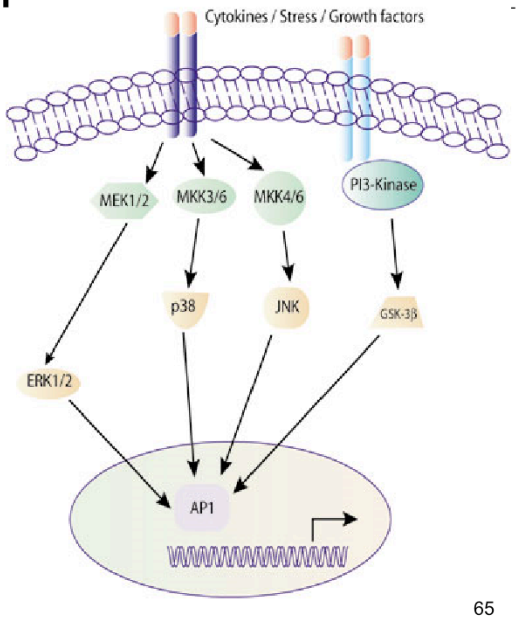
And these interactions mostly involves gene regulatory circuits, justified a stochastic approach to account for randomness. Stochastic processes in gene expression can be exploited by cells to facilitate fate decisions including differentiation.

These interactions also requires computational tools (bifurcation discovery) that can help find parameter settings that allow switching.

Mediators of the B Cell Differentiation Program (Plasma Cell)



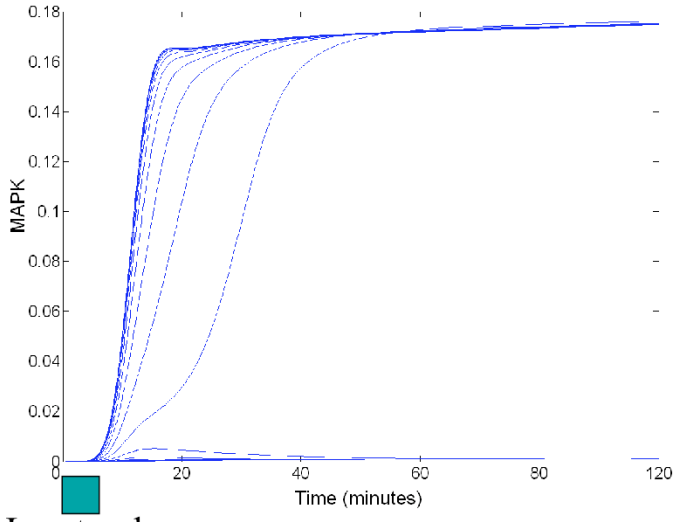
AP-1 activation



http://www.dartmouth.edu/~brenner/gene144-06/wasiuk.html

65

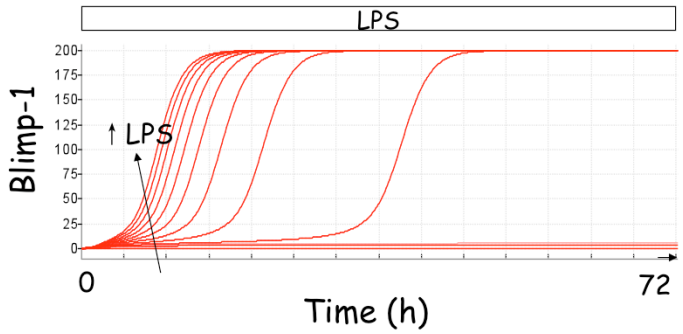
MAPK time-course and bifurcation after a short pulse of PDGF



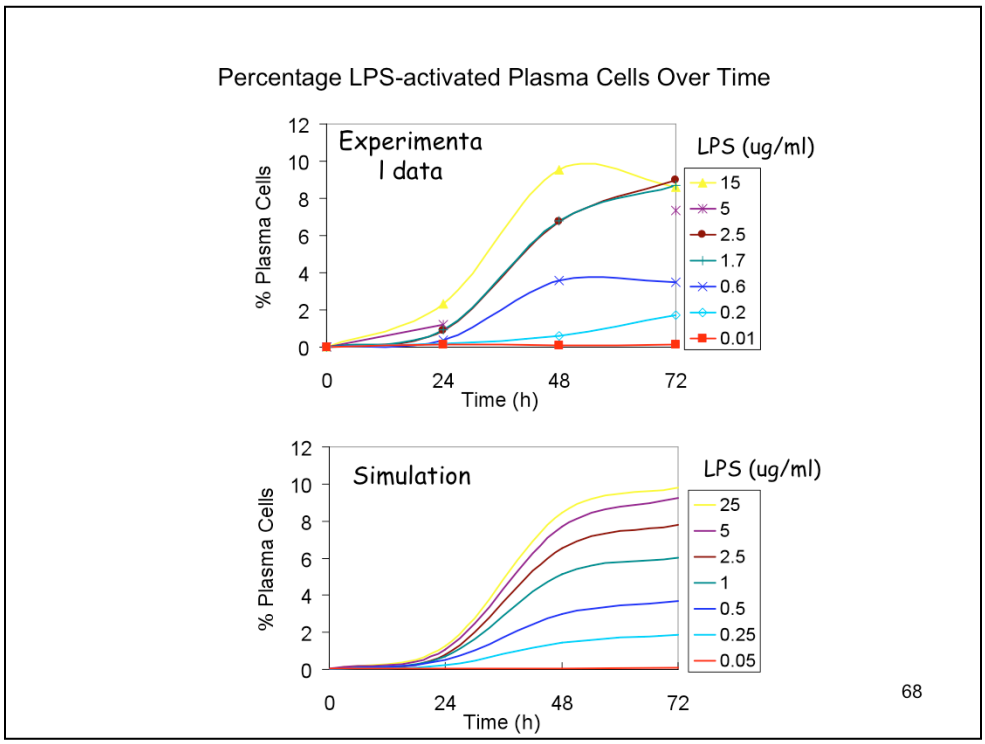
Input pulse

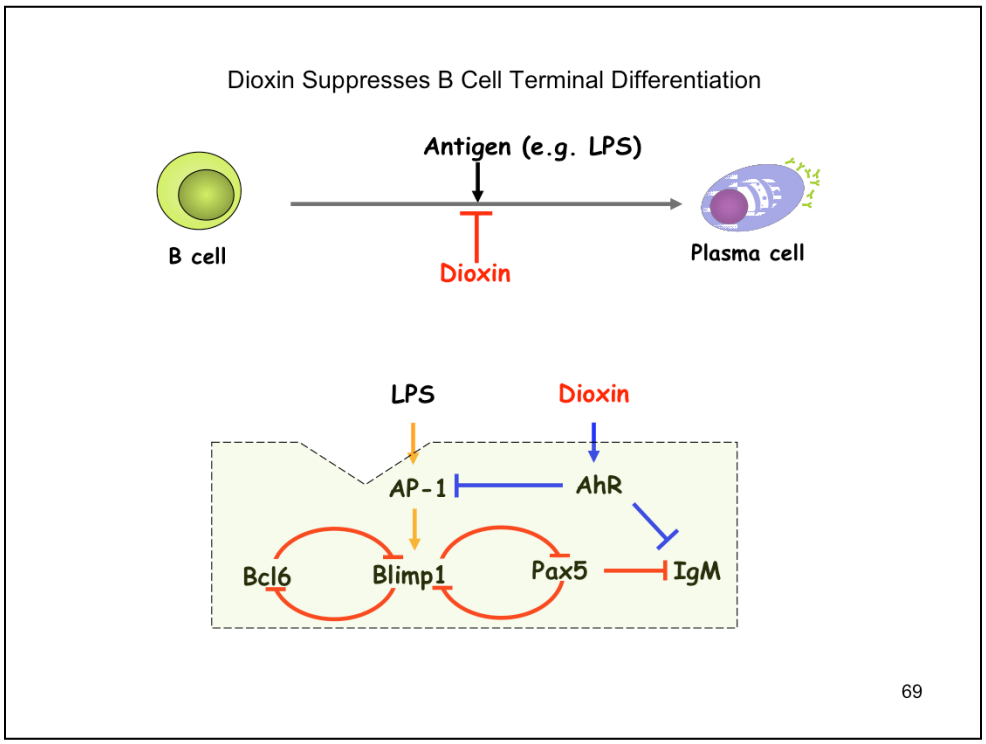
66

Prediction: LPS activates Bcl6-Blimp1-Pax5 switch with a threshold dose



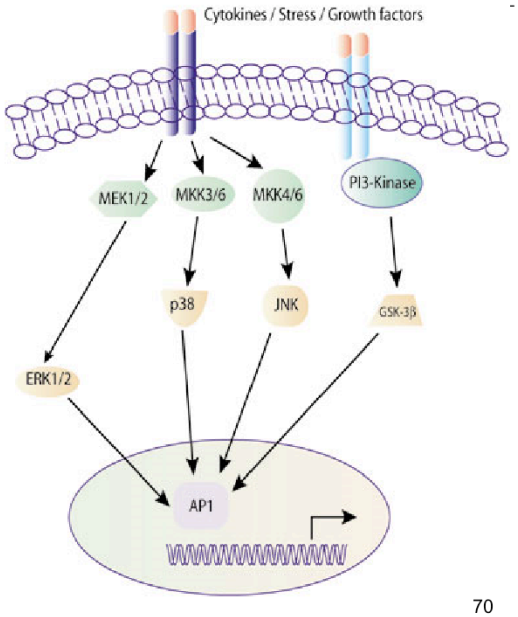
67


Percentage LPS-activated Plasma Cells Over Time
Experimenta
l data
% Plasma Cells
Time (h)
LPS (ug/ml)
15
5
2.5
1.7
0.6
0.2
0.01
Simulation
% Plasma Cells
Time (h)
LPS (ug/ml)
25
5
2.5
1
0.5
0.25
0.05
68



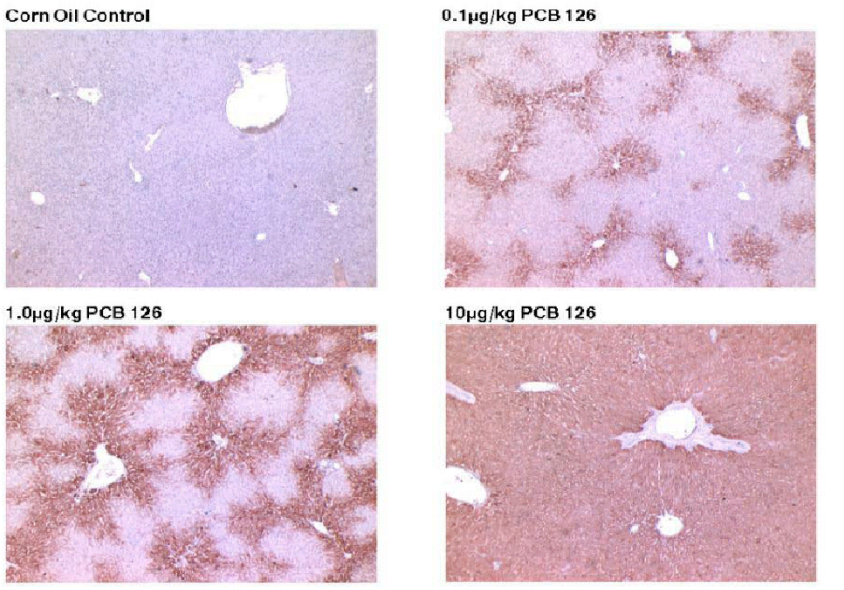
If this bistable is what controlling the transition from B cell to plasma cell, then dioxin should impinge upon it.

TCDD and
AP-1 activation,
appropriate kinetic
description?



70

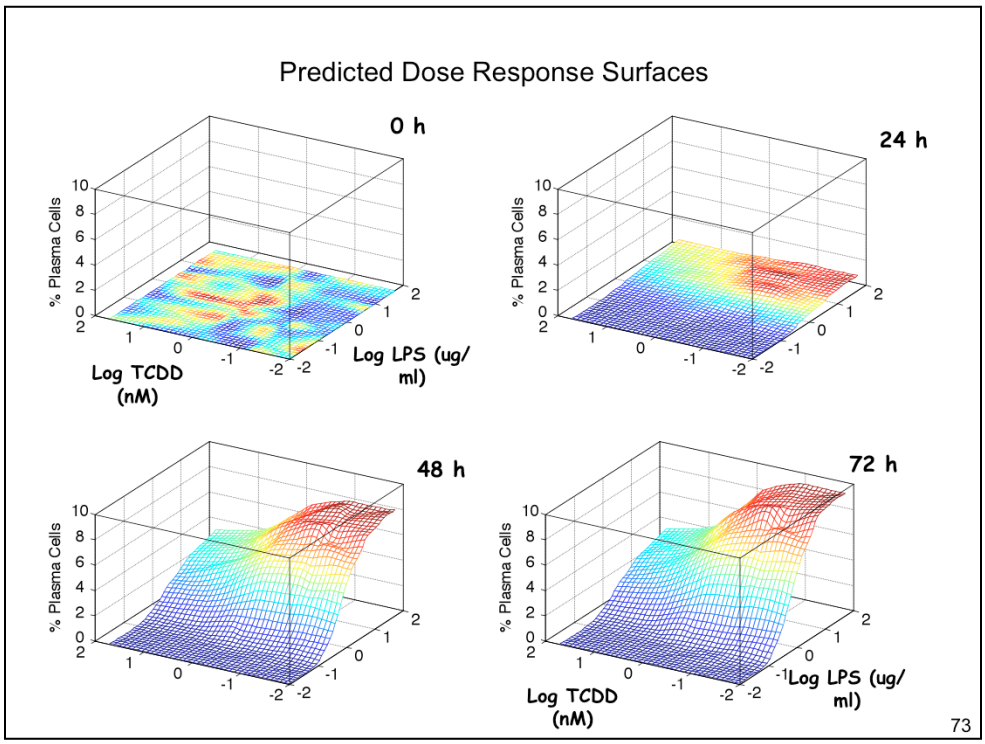
http://www.dartmouth.edu/~brenner/gene144-06/wasiuk.html


Corn Oil Control
0.1µg/kg PCB 126
1.0µg/kg PCB 126
10µg/kg PCB 126
71

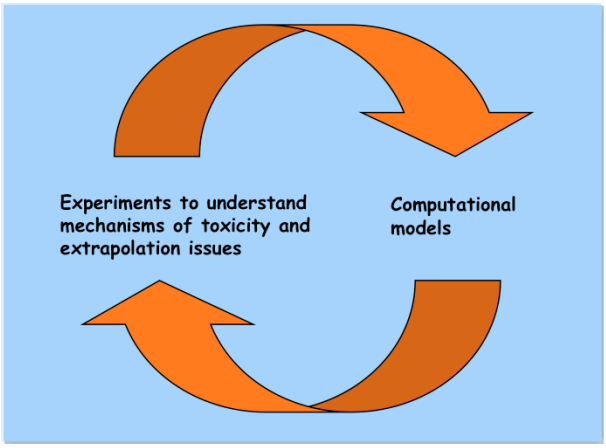
Research needs

- Fuller mapping of TCDD-mediated signaling
- How does TCDD affect gene expression?
  - Gas pedal or light switch?
- All doable with existing technology!

72


Predicted Dose Response Surfaces
0 h
24 h
48 h
72 h
% Plasma Cells
Log TCDD (nM)
Log LPS (ug/ml)
73

Iteration between the laboratory and computational modeling



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Summary

- Dose-response is a function of biological mechanisms.
- Computational models of these mechanisms improve the efficiency of research and provide the capability for prediction.
- Need quantitative understanding of how TCDD-AhR interacts with AP-1 and other sites in the B cell differentiation program

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Qiang Zhang

Sudin Bhattacharya

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End

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http://tools.niehs.nih.gov/srp/risk_elearning/

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