# Molecular Circuits, Biological Switches, and Nonlinear Dose–Response Relationships

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**Signaling motifs (nuclear transcriptional receptors, kinase/phosphatase cascades, G-coupled protein receptors, etc.) have composite dose–response behaviors in relation to concentrations of protein receptors and endogenous signaling molecules. "Molecular circuits" include the biological components and their interactions that comprise the workings of these signaling motifs. Many of these molecular circuits have nonlinear dose–response behaviors for endogenous ligands and for exogenous toxicants, acting as switches with "all-or-none" responses over a narrow range of concentration. In turn, these biological switches regulate large-scale cellular processes, e.g., commitment to cell division, cell differentiation, and phenotypic alterations. Biologically based dose–response (BBDR) models accounting for these biological switches would improve risk assessment for many nonlinear processes in toxicology. These BBDR models must account for normal control of the signaling motifs and for perturbations by toxic compounds. We describe several of these biological switches, current tools available for constructing BBDR models of these processes, and the potential value of these models in risk assessment.** ***Key words:*** **biological switches, dose–response relationships, endocrine-active compounds, estrogen, molecular circuitry, pharmacodynamic models, risk assessment, TCDD.** *Environ Health Perspect* **110(suppl 6):971–978 (2002).**
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Risk assessment approaches increasingly use two complementary concepts—mode of action and tissue dose—to organize available toxicological and epidemiological studies, to decide on the shape of the dose–response curve (linear, nonlinear, or threshold), and to conduct low-dose, interspecies, dose route, and interindividual extrapolations (Andersen and Dennison 2001). Mode of action provides a basis to decide qualitatively on the shape of the dose–response curve (U.S. EPA 1999). By knowing the nature of tissue dose, i.e., whether it is parent chemical, metabolite, receptor-bound toxicant, etc., physiologically based pharmacokinetic (PBPK) models can be used to calculate tissue dose metrics for various dose routes, species, and dose levels to support extrapolations (Clewell and Andersen 1985). These two organizing principles, mode of action and tissue dosimetry, have the potential to encourage application of a wide array of mechanistic data and biologically based modeling in chemical risk assessment. However, for nonlinear and threshold responses, acceptable exposure limits are still primarily derived by a procedure based on objective evaluation of dose–response curves, followed by application of multiple uncertainty factors that adjust for interspecies differences in response, for interindividual response differences in humans, for adequacy of available data, and to adjust to lifetime exposure periods. Many of these uncertainty factors are used because of ignorance about the shape of dose–response curves at low incidence levels for these responses.

A variety of toxicants interfere with cellular signaling by endogenous endocrine system hormones and biological receptors (Kavlock and Ankley 1996). Our understanding of these signaling pathways, at least qualitatively, has increased markedly in recent years through new techniques in molecular biology, including studies using knockout and transgenic animals and the development of high throughput methods in genomics, transcriptomics, and proteomics. Some major signaling motifs that are of interest for toxic responses include actions of nuclear receptors, such as members of the steroid hormone family receptors (Landers and Spelsberg 1992), and G-protein–coupled cell-surface receptors, such as those proteins that recognize peptide-stimulating hormones secreted by the anterior hypothalamus (Clement et al. 2001). A possible impediment to the quantitative application of these data in risk assessment is the sheer volume of the information being collected at different levels of biological detail, i.e., the molecular, cellular, organ system, organism, and population levels. How will we order and make sense of all this information to provide a more complete understanding of dose–response curves for endogenous signaling components and for exogenous compounds that interfere with these signaling motifs?

Noble recently emphasized the complementary roles of observation and computational modeling in studying the physiological function of biological systems. He writes:

> The amount of biological data generated over the past decade by new technologies has completely overwhelmed our ability to understand it . . . Indeed, it is hard to see how [the] unraveling of complex physiological processes can occur without the iterative interaction between experiment and simulation . . . In a few years' time we shall all wonder how we ever managed to do without [computational models]. . . . (Noble 2002).

These same comments apply to the generation of information on mode of action in chemical risk assessment. Studies on mode of action are essentially qualitative in nature and must be organized by quantitative computational models to make predictions of the shape of the dose–response curves and to suggest important new experimentation. In risk assessment these computational models are referred to as biologically based dose–response (BBDR) models and provide the substrate for simulations that link mode of action research with predicted physiological consequences of exposures (Setzer et al. 2001). Here we discuss risk assessment approaches for nonlinear toxicological processes that take into account dose–response behaviors of native signaling molecules required for normal function, perturbations of these systems by the presence of toxicants, and BBDR modeling of the underlying molecular circuitry associated with normal and impaired function of these signaling motifs.

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## Perturbations of Signaling Pathways

### Molecular Circuitry

Gene and protein arrays, simultaneously assaying the expression of hundreds or thousands of genes and proteins, provide information on expression of suites of genes and batteries of protein products that are coordinately regulated and the manner in which toxicants alter their expression. However, the data from these arrays must be integrated to provide an understanding of biological function rather than serving simply as a catalog of change. Lander and Weinberg have expressed their opinion regarding the overall goal of genomics:

> The long-term goal is to use this information to reconstruct the complex molecular circuitry that operates within the cell to map out the network of interacting proteins that determines the underlying logic of various cellular biological functions including cell proliferation, responses to physiologic stresses, and acquisition and maintenance of tissue-specific differentiation functions. A longer term goal, whose feasibility remains unclear, is to create mathematical models of these biological circuits and thereby predict these various types of cell biological behavior. (Lander and Weinberg 2000)

The underlying concept here is that biological functions require the successful operation of specific circuits that coordinate information flow and govern cellular behavior under a variety of physiological conditions. Analogous to electric circuits, molecular circuits consist of components (proteins, RNAs, signaling molecules, etc., and cellular targets) that organize flows of cellular information, although there is arguably more variability in the biological than in the electrical system. Toxicants, especially those that interfere with hormones and signaling motifs, can interfere with normal functioning of these molecular circuits, leading to altered function and ultimately to toxicity.

## Biological Switches

Molecular circuits are controlled by energy provided in the form of receptors and ligands that activate signaling motifs, leading to downstream biological consequences. Most responses to these signaling compounds are themselves nonlinear. The process by which some endogenous ligands or toxicant compounds cause nonlinear responses is referred to here as "switching." Switches activate fundamental changes in functional behaviors of cells in an all-or-none fashion. The more nonlinear the response is, the more switch-like it is. Switches are another functional component of circuits. Signaling motifs with linear dose–response behavior would be expected to show graded responses to changes in signal concentration. However, very few examples of graded transcriptional response have been reported for eukaryotic gene expression (Louis and Becskei 2002). The carefully timed development of organisms from a single fertilized cell requires coordination of a series of cellular switches to complete the transformation from a single fertilized cell to a mature organism (Davidson et al. 2002).

Several nonlinear switching processes have been examined quantitatively. Progesterone induces maturation of *Xenopus* oocytes. The dose response for maturation of individual oocytes was described with a Hill equation for the activation of mitogen-activated protein kinase (MAPK) (Ferrell and Machleder 1998):

$$\text{Response} = \frac{\text{Response}_{MAX} \times C^n}{K_d^n + C^n}, \quad [1]$$

where $C$ is concentration of progesterone and $K_n^d$ is the concentration at half-maximal response. In the Hill equation the exponent $n$ (Hill coefficient) determines the steepness of the dose–response curve. In simpler molecular systems such as dimerization of estrogen receptors, an $n$ value of 2.0 for a functional response such as gene expression may simply indicate involvement of a bimolecular process. With oxygen binding to hemoglobin, $n$ values of 3–4 indicate that binding of the first oxygen molecule to the hemoglobin tetramer leads to structural changes, facilitating binding to the remaining three sites (allosteric binding). With *Xenopus* oocyte maturation, Hill coefficients for individual oocytes were reported to be between 20 and 30 (Ferrell and Machleder 1998). These high values indicate an all-or-none switch for maturation in any individual oocyte. For populations of oocytes the progesterone concentrations causing maturation were distributed fairly broadly. Evaluation of populations of oocytes did not show the all-or-none responses noted in individual cells. In the oocyte, progesterone receptor and MAPK cascade-signaling motifs combine to control a switch that initiates maturation circuitry.

Receptor upregulation may also produce nonlinear biological responses. Certain bacteria change phenotypic characteristics under conditions that encourage bacterial growth. As bacterial concentrations increase, secondary metabolites are released into the surrounding media. As the concentration of this signaling molecule increases, the metabolite, in concert with a cytosolic receptor, initiates transcription of a set of genes leading to new phenotypic characteristics in the bacteria. *Photobacterium fischreii* regulates genes that control phosphorescence via this mechanism (Fuqua et al. 1994). The suites of genes controlled by the secondary metabolite–receptor interaction include the receptor protein itself and an enzyme that converts the secondary metabolite to a higher-affinity ligand. Thus, these metabolites indirectly serve as surrogates for the concentration of bacteria. Because of the relationship to bacterial number, these responses are called "quorum sensing." The metabolite accumulation signals the bacteria that they are present in sufficient quantity to change phenotypic characteristics. Many bacteria, including film formation in some species (Costerton et al. 1995; Davies et al. 1998), use quorum-sensing motifs to respond to environmental stimuli. Efforts to model these biological processes are also under way (Koerber et al. 2002).

Estrogen receptor upregulation controls vitellogenesis in some fish, leading to nonlinear dose–response relationships and stable differentiation of hepatocytes treated with high doses of estrogenic compounds (Shapiro et al. 1989). The possible role of receptor upregulation in these nonlinear responses was investigated by simulation with a pharmacodynamic gene induction model. Several generic gene induction models were developed and exercised (Andersen and Barton 1999). These models recapitulated nonlinear behaviors with very high Hill coefficients. More recently a transcriptional switch controlling methylation of nucleosomes and transcriptional cofactors was described (Xu et al. 2001) that was associated with coactivator-associated arginine methyltransferase-1 (CARM-1). This molecule acts as a coactivator for nuclear hormone signaling via histone methylation and as a co-repressor of cyclic-AMP–associated signaling pathways. This switch involves limiting concentrations of the CARM-1 coactivator and acts via histone modification to increase access to specific genes and promoter regions. Activation of transcription by the nuclear receptors, in concert with CARM-1, is expected to activate groups of genes and to silence others.

The proper function of all these signaling pathways requires correct concentrations of a variety of endogenous proteins and signaling molecules. To a very large extent, dose response, a concept used frequently in toxicology in regard to adverse responses, now has an equally important position in normal biology. The ideas expressed in this regard are proper gene dosages in cells, leading to proper concentrations of receptors and ligands for normal function. The proper functioning of molecular circuits and maintenance of healthy conditions in the organism requires appropriate doses of various signaling components and

their presence at appropriate times for activation of biological switches.

### Endocrine-Active Compounds

Many endocrine-active compounds (EACs) interact with the normal cell circuitry to mimic or antagonize the actions and functions of normal signaling systems. Excess of EACs or deficiencies of natural hormones alter hormonal system function, leading to impaired health. Impaired health covers a wide range of responses, including loss of viability, impaired performance, altered reproductive success, and delayed maturation. The actions of many signaling elements—cell-surface receptors, cytosolic transcriptional factors, kinase/phosphatase cascades—are now more completely understood than just a few years ago. Studies of the toxic responses to EACs need to begin with examination of normal function of these signaling motifs and how the normal function becomes perturbed by exogenous compounds (Figure 1). The first requirement in a BBDR model, then, is to develop an adequate representation for the dose–response control of normal function. Secondarily, the focus is on the perturbation of normal function by exogenous compounds. This reorientation to a perturbation approach to normal biology rather than an emphasis on final pathology provides new avenues and strategies to evaluate dose–response relationships in biology and in toxicology. These EACs serve as examples through the remainder of this article.

## BBDR Models for Molecular Circuits and Switches

### Concerted Cellular Responses

Most dose–response assessment models in toxicology assume smooth, continuous changes in response to dose. These models describe many chemical processes by statistical methods with average behaviors of molecules, as the numbers of particles involved in most reactions and interactions are very large. The real world of cells demonstrates a more complex variety of interacting circuitry. At the cellular level, behaviors are more likely to be nonlinear and stochastic. A cell either divides or it does not divide. In moving from one phenotypic state to another, all the components have to change in concert to achieve a smooth pleiotropic alteration in cell characteristics. A challenge in formulating the mathematical models of cellular functions is the requirement to grasp the manner in which continuous changes of chemical variables (i.e., ligand and receptor concentrations) lead to stochastic responses such as apoptosis, proliferation, differentiation, or activation of global cellular circuitry by exposure to chemicals.

Stochastic, nonlinear models of cellular-level responses may provide the basis for developing tools that will simulate nonlinear dose–response behaviors toward toxic exposures. Some stochastic models assess cancer risks based on rates of cell division, cell death, and cell mutation. The Moolgavkar-Venzon-Knudson (MVK) model (Figure 2) represents a stochastic model of carcinogenesis (Moolgavkar and Knudson 1981). These cancer models have to be initially set to describe tumor incidence in the control animals. These background rates are then altered, i.e., perturbed by the actions of toxicant compounds. In developing BBDR models it is necessary to evaluate the effect of dose on intrinsic biological parameters of the model. The effects can be described empirically, as has usually been done, or mechanistically. For the cancer models the stochastic aspect involves some probability of division, death, or mutation that occurs randomly. Mechanistically, the requirement is to understand (model) the relationship of these probabilities with dose and to describe the manner in which dose changes the probability of division, death, or mutation during a time interval. The relationships between dose and cell proliferation or between dose and cell apoptosis are unlikely to be simple continuous functions. The control of biological circuitry and the transition between different states of the cellular circuitry in response to exogenous signaling molecules should determine the dose–response manifestations for proliferation, apoptosis, and mutation in many of these cancer models.

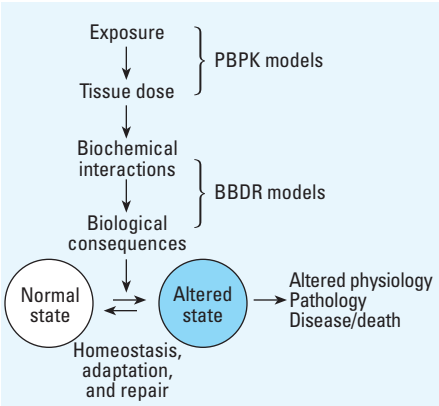


**Figure 1.** Dose–response models for perturbations of signaling motifs focus on normal biology including dose–response behavior for endogenous signaling molecules and cognate receptors. The actions of EACs would appear as perturbations on the normal, nonlinear control of molecular circuitry and the switching modules moving between and among various circuits.

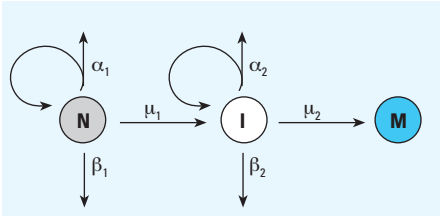


**Figure 2.** The MVK model for cancer, including cell division, cell death, and probabilities of mutation during replication. Although the model itself is stochastic, the biological processes represented by birth and death may themselves represent toxicant actions on nonlinear signaling motifs associated with perturbation of cellular circuits by the presence of toxicants. *N* represents the population of normal cells, *I* the initiated cells, and *M* the mutated cells. The parameter α represents the cell birth rate, β the cell death rate, and μ the transformation rate, where subscript 1 refers to the normal population and 2 refers to the initiated population.

### Receptor-Mediated Control of Gene Products

Many EACs directly or indirectly interfere with gene expression. In discussing molecular circuits the changes are generally coordinate alterations in groups of genes that lead to altered biological characteristics of the affected cells. It is often possible to measure responses of single genes with great precision using modern techniques such as polymerase chain reaction amplification of gene transcripts. Molecular markers, such as induction of cytochrome P4501A1 (CYP1A1) message by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) (Vanden Heuvel et al. 1994), allows observation of the dose–response curves in lower dose ranges than possible when examining overt adverse responses of the organism. However, these measurements lead to questions about the linkage between these precursor effects and clearly adverse responses of the organism. For instance, should the observation of a 1% increase in CYP1A1 mRNA after 2,3,7,8-TCDD treatment be considered adverse? This focus on a single gene may not be the correct one for assessing toxicity. Dioxin and similar receptor-mediated EACs do not simply control expression of a single gene in the intact liver. They alter concentrations of a battery of gene products to induce a concerted, pleiotropic response in hepatocytes (Bock 1993).

### An Example with Tumor Promotion

Dioxin is a liver carcinogen in rats and a tumor promoter (Kociba et al. 1978; Pitot et al. 1987). Many liver tumor promoters act by transiently increasing proliferation of hepatocytes with longer-term adaptation to the exposures. The adaptation, with phenobarbital, involves elaboration of transforming growth factor-β, a specific growth factor that constrains hepatocyte proliferation (Jirtle et al. 1991). Cells resistant to

cytoinhibition are presumed to derive a growth advantage and grow out to preneoplastic foci under the selection pressure from the promoter. The dose–response relationship for carcinogenesis requires characterization of the dose of promoter required to increase the proliferation of hepatocytes.

Phenobarbital, in common with a large number of liver tumor promoters, has a receptor-mediated mode of action. These promoters interact with protein receptors that serve as transcriptional modulators to alter expression of batteries of genes in the hepatocytes. Phenobarbital interacts via the constitutive androstane receptor (Waxman 1999). Other liver tumor promoters act via the aryl hydrocarbon receptor (AhR) (Wilson and Safe 1998), the peroxisome proliferation–activating receptor (Weghorst et al. 1994), or the pregnane-X receptor (Waxman 1999). All these receptors, in concert with the toxic compounds, act to increase expression of batteries of genes, leading to several alterations in expression of many individual gene products, including specific genes. The effects on cell-level characteristics are likely to be associated with these pleiotropic responses.

Among these promoters, 2,3,7,8-TCDD has received a great deal of attention in the past decade as the U.S. Environmental Protection Agency has reevaluated the risks of exposure to this environmental contaminant (U.S. EPA 2000). PBPK and protein induction models describe dioxin kinetics in the body, including binding to the AhR with activation of specific gene products (Kohn et al. 1993; Leung et al. 1990). These models have also described the induction of specific genes through interactions of the AhR–TCDD complex with DNA-response elements for the AhR and various partnering molecules (Andersen et al. 1997b. Like most mathematical models of biological systems, the BBDR models presently available for dioxin represent a significant simplification of the individual molecular processes. Simplifications are necessary to attain a computationally tractable model and may be useful to gain insights about dose–response behavior (Suk and Yang 2002). Bailey has emphasized the value and necessity of using simplifications in modeling complex, biological systems (Bailey 2001). However, it is important that the simplifications retain the important biological aspects of the responses.

The regulation of gene expression by dioxin and dioxinlike polychlorinated biphenyls (PCBs) and the AhR has been investigated in a variety of systems, including many cell constructs with the CYP1A1 promoter upstream of particular marker genes (Garrison et al. 2000; Jeon and Esser 2000). Such constructs allow evaluation of the components necessary to control gene expression by the AhR in a system with an available promoter. However, in the intact animal, the silencing or activation of genomic structures that are not present in the cell constructs may alter gene expression. We are studying AhR-mediated induction of CYP1A1 in primary hepatocytes. Induction does not follow coherent dose–response relationships expected for a Hill relationship with a low *n* value. This behavior is true both *in vivo* (Chubb et al. 2002) and *in vitro* in the primary hepatocytes (French et al. 2002), as shown in Figures 3 and 4, respectively.

With AhR agonists, the response of cells in the liver and of cells *in vitro* does not appear to follow a continuous response pattern where a 50% induction in the total liver is reflected by a 50% induction in all hepatocytes. The induction of individual cells appears to occur almost in an all-or-none fashion. Cells are either induced or remain in a basal state (Andersen et al. 1997a; Tritscher et al. 1992). This response, a concerted response of a cell to the receptor–ligand complex, is not yet well understood. The molecular circuitry that causes this switchlike behavior leads to a qualitative alteration in response over a narrow range of dose, moving the cell from one state to a new one. In the liver different acinar regions have varying sensitivity for induction. At low doses centrilobular cells are induced. As dose increases, more of the cells in the liver become induced and the region of induced cells progresses toward the periportal area of the liver acinus (Figure 3). Andersen et al. modeled regional enzyme induction using a semiempirical induction model (Andersen et al. 1997a) that could represent the differential induction throughout the liver (Figure 5). This regional induction model coupled a PBPK model for the disposition of dioxin in the body, a geometric representation of the

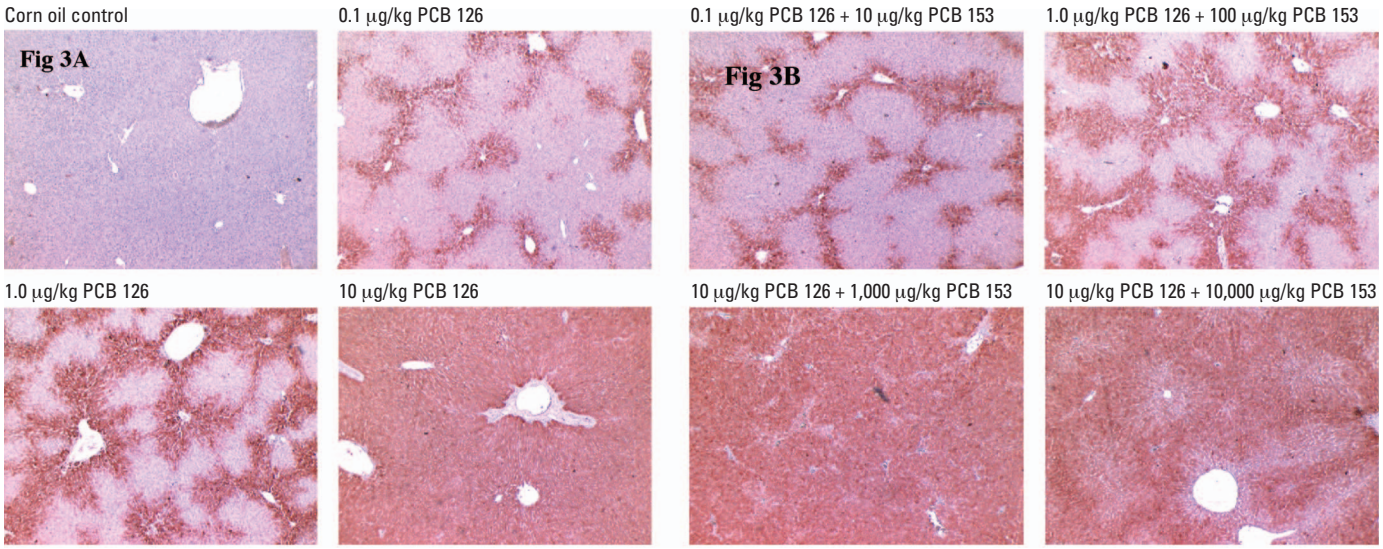


**Figure 3.** Induction of CYP1A1 and 1A2 by PCB 126 (3,4,5-3′,4′-pentachlorobiphenyl) in rat liver and the influence of a second promoter. (*A*) The pattern of immunohistochemical staining for CYP1A1 protein is consistent with a switch that induces a change from a normal phenotype to an AhR-responding phenotype over a narrow range of dose. In the context of the topic of this article, this AhR-agonist PCB has altered the molecular circuitry of these hepatocytes, leading to activation of a cellular switch. (*B*) In the presence of high daily doses of PCB 153, a phenobarbital-like enzyme inducer that increases concentrations of a different cytochrome (CYP2B1/2), PCB 126 no longer induces cells in the centrilobular region of the liver. The presence of high PCB 153 (10,000 μg/kg) has turned off the AhR switch (Chubb et al. 2002).

liver acinus, and a more empiricalal description of enzyme induction. Enzyme induction in each zone of the liver acini was modeled with a Hill-type equation, with variable affinities for AhR–ligand–DNA interactions in each acinar region. Successful modeling of induction in a five-compartment liver acinus required that binding affinities differ by a factor of three between adjacent acinar regions, with high Hill coefficients for induction (i.e., 4–5) in each region of the acinus. The next step will be to provide a more mechanistic description of the molecular circuit and switching that comprises this all-or-none behavior.

These switching responses of hepatocytes appear to represent a reversible differentiation to a new stable state (a new phenotype) of the hepatocyte. This differentiation includes the concerted induction of a battery of genes. Interestingly, the current induction models (Andersen et al. 1997b; Kohn et al. 1993), if extended to describe multiple genes with independent promoters, would give rise to competitive, not cooperative, interactions. Biological mechanisms that might explain nonlinear, concerted responses of gene batteries include receptor autoregulation, genomic level switches, such as that noted for histone methylation, or kinase cascades. We are now developing an experimental system to study responses of isolated hepatocytes to dioxinlike compounds (French et al. 2002). This experimental system is intended to permit evaluation of the mechanistic characteristics of the hepatocyte switch, including the role of kinase cascades or histone modification, in these processes. The accumulation of more mechanistic data on induction is necessary to provide sufficient biological detail to predict low-dose behavior.

## Discussion

### Modeling Tools for Describing Biological Switches

***Chaos and attractors.*** Our continuing evaluation of induction responses of hepatocytes has led us to a set of new concepts for future exposure dose–response assessments. They include biological switching, molecular circuits, and multiple stable states of the cells, in addition to our old concerns regarding the relationship of molecular-level responses and the ultimate expression of toxicity. How will we model these responses to predict responses over a wide range of dose based on biological characteristics of cellular switches? Chaos and complexity theorists have discussed concepts of stable attractors in complex systems. In the context of molecular biology, an attractor is the proteomic state of the cell (including the antecedent genomic state) that is stable because of its ability to maintain homeostasis within a range of conditions. The attractor concept implies that there are a finite number of stable states that exist rather than a smooth transition between infinite numbers of cell phenotypes. In particular, these concepts suggest that mammalian cells may exist in a suite of differentiated forms that represent stable attractors for the overall behavior of the genetic content of the cell (Kaufman 1995). Shapiro and colleagues (1989) and Simon et al. (1988) have pursued limited modeling of stable states for hormone responsiveness of cells for estrogen-responsive actions. The basal or induced states in hepatocytes caused by tumor promoters may represent two stable attractors. The increasing concentration of the receptor–ligand complex may alter the concentration of a limited set of initial gene products that move the circuitry from that for one stable attractor to a second stable attractor. Over time, the overall content and behavior change, consistent with the new stable state. The new state determines the pathological or physiological consequences of induction for the cell, whereas the dose response of the process is more likely determined by some of the early interactions of the ligand and the receptor molecules in the most sensitive population of cells. Tyson et al. (2001) have used similar cellular paradigms to describe the cell cycle.

***Early (transient) and late (persistent) responses to signaling molecules.*** Switches are likely to be organized by positive feedback circuits to drive transitions from one state to another. In normal maintenance of cell function in a given state, homeostatic responses are more generally associated with negative

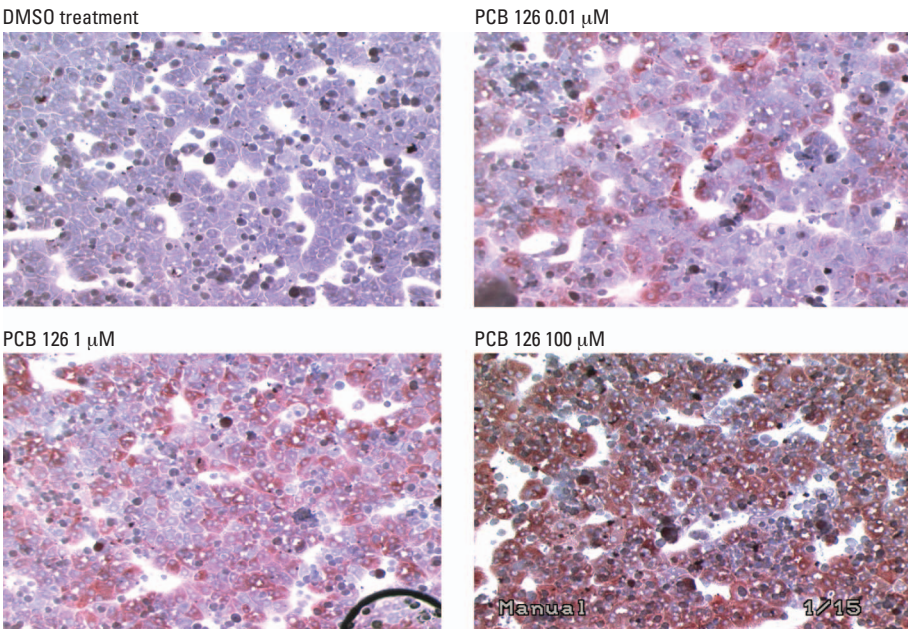


**Figure 4.** CYP1A1 staining *in vitro*. Immunohistochemical staining for CYP1A1 in rat primary hepatocytes treated with various concentrations of PCB 126 for 24 hr. The staining occurs in increasing numbers of cells with increasing dose rather than increasing concentrations of protein in each cell proportional to dose (French et al. 2002).

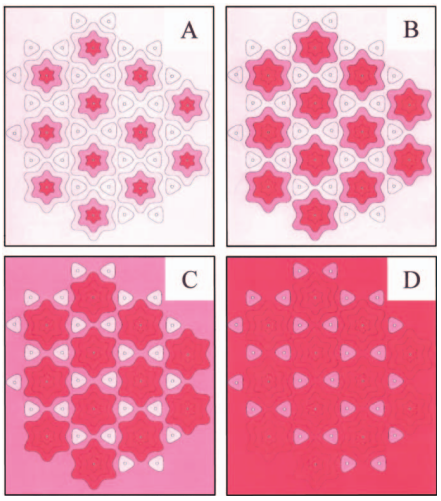


**Figure 5.** The predicted staining pattern for dioxin induction of CYP1A1 in rat liver at various doses of dioxin. A PBPK model was linked to a nonlinear, semiempirical model of gene induction to examine the degree of nonlinearity indicated by the regional induction data with various daily doses of dioxin, as observed by Tritscher et al. (1992). The regional Hill coefficient for protein induction required to provide demarcation between adjacent regions of the liver, 4–5, indicated a switch controlling different phenotypic behaviors of the hepatocytes. Reproduced from Andersen et al. (1997a) with permission from Academic Press.

feedback, as with the feedback processes for endocrine target-organ function and release of stimulating hormones from the pituitary. One concept involved in steroid hormone function (Landers and Spelsberg 1992) and in memory storage was the involvement of early and late responses organized by transcriptional receptor or nervous system activation of cells (Kandel 2001). Here, early responses are more transient; however, if these signals persist or are of sufficient magnitude, they initiate more permanent alterations of genetic expression of gene batteries and alterations of cell characteristics.

***Some possible experimental and computational models.*** Among a wider range of possibilities, it is apparent that nonlinear switching modules exist for receptor autoregulation (Shapiro, et al. 1989), kinase/phosphatase cascades (Ferrell and Machleder 1998), and $Ca^{2+}$-mediated nerve-cell signaling related to long-term potentiation (Bhalla and Iyengar 1999). Although the nonlinear characteristics of these switches are evident, none have been examined in sufficient detail to provide a quantitative understanding of the molecular basis of the switch and its influence on the dose–response curve at low incidence levels. Bhalla and co-workers have modeled various cascade interactions that may be involved in long-term potentiation in neurons and maintaining memory. Their work included biologically realistic kinetic models of these processes that capture the emergence of altered cellular characteristics arising from particular pulse trains at the cell surface (Bhalla and Iyengar 1999). Their model structures and other efforts to create virtual cells should aid in providing the biological detail for realistic BBDR models for various signaling motifs.

Glycoprotein-stimulating hormonessuch as thyroid-stimulating hormone, follicle-stimulating hormone (FSH), and luteinizing hormone are released by the pituitary and have end-organ effects on endocrine tissues. These hormones cause cellular responses by binding to cell-surface G-protein–coupled receptors. Binding leads to activation of adenylate cyclase (AC), with production of cyclic adenosine monophosphate (cAMP). Phthalate esters interfere with FSH-mediated signaling in Sertoli cells (Heindel and Chapin 1989), although the exact sites of interaction remain uncertain. The responses of various endocrine tissues to these hormones depend on cAMP and on a variety of other signaling molecules in the cell (Richards 2001), including inducible kinases and guanosine triphosphatases. The efforts to unravel these signaling pathways should lead to a representation of the key functional elements involved in G-protein–coupled signaling in these cells and an improved understanding of the role of these cascades in toxicity, disease, and health.

***Generic tools.*** New methods for modeling the control of gene batteries in normal systems may use Boolean networks (Kaufman 1995) or apply neural network models (Vohradsky 2001) for expression of multiple gene families. Quantum computational or predictive structural activity relationship approaches, such as the reaction network modeling approach being developed in our laboratory (Liao et al. 2002), should facilitate the simulation of molecular circuits and cellular switches. The contributions from groups developing more quantitative tools to assess physiological coordination of multiple cellular activities, e.g., the Physiome Project (Physiome 2002), may significantly expand the mathematical dose–response modeling approaches used by toxicologists and risk assessors for integrating the exposure–response–dose paradigm into a perturbation paradigm assessing toxic potential of compounds. Programming tools for modeling neuronal function (Genesis 2002; Wilson et al. 1989) or the entire cell (Tomita et al. 1999) are also available for evaluating integrated signaling motifs. Although there are many candidate tools for developing models of genetic circuitry, the immediate future in this area will require trial and error to discern the mathematic/simulation tools that will allow the most rapid progress. A brief synopsis of some of the major signaling motifs and several available tools for analysis of biological circuits is listed in Table 1.

***Systems theory.*** Increasingly, integrative biological research relies on systems theory for connecting biological experiments with computational descriptions of cellular structure and dynamics. Systems theory, i.e., the study of systems that are conceptualized using networks to define information flows, uses engineering concepts such as robustness, fragility, and failure cascades (Csete and Doyle 2002) and serves as an organizational scaffold to support complex system modeling. Current interest in systems biology is partly an outgrowth of the need to conceptualize, hypothesize, modularize, design experiments, communicate ideas, and share results between research institutions. It is also partly a result of the need to integrate new computational methods, to construct and interface complex models from different sources and disciplines, and to integrate diverse information from genomics, proteomics, and metabonomics into coherent models readily shared among different research groups. Bifurcation theory (Tyson et al. 2001) may serve as a computational aid to identify attractors, even though the parameter space for the individual components of the biological system becomes

**Table 1.** Some signaling motifs and possible analysis tool.

| Motif or tool | Mechanism | Reference |
|---|---|---|
| Empirical transcriptional activation models | Enzyme induction, receptor binding and transcriptional upregulatory mechanisms | Kohn et al. 1993<br>Andersen et al. 1997a,b |
| Steroid hormones | Vitellogenesis | Shapiro et al. 1989<br>Tata et al. 1993 |
| | Oocyte maturation | Ferrell and Machleder 1998 |
| | Early/late responses | Landers and Spelsberg 1992<br>Schuchard et al. 1993 |
| Peptide hormones | FSH-and G-protein–coupled surface receptors | Clement et al. 2001 |
| Cell cycle | Positive feedback in protein regulation through phosphorylation and related control mechanisms | Tyson et al. 2001 |
| | Cell cycle map | Kohn 1999 |
| DNA methylation | Transcriptional regulation through histone modification | Xu et al. 2001 |
| Cellular signaling | Alliance for cellular signaling | AFCS 2002 |
| Stochastic processes | Two-stage tumorogenesis | Moolgavkar and Knudson 1981 |
| | Two-stage growth model for precancerous lesions and tumors | Conolly and Kimbell 1994 |
| Neuronal function | Genesis | Genesis 2002 |
| Systems biology | Systems Biology Markup Language | Systems Biology 2002 |
| Modeling tools | Berkeley Madonna | Macey and Oster 2002 |
| | Advanced Continuous Simulation Language (ACSL) | ACSL 2002 |
| | MatLab | MathWorks 2002 |
| | Scope | SCoP 2002 |
| | BioSpice (under development) | Biospice 2002 |

very large. This approach examines the effects of parameter variation on solutions of the nonlinear circuit model.

Rapid progress applying systems biology is apparent in a number of research fields, including immune function (Germain 2001), the cardiac system (Noble 2002), developmental biology (Davidson et al. 2002), and prokaryotic systems (Kitano 2002; Weng et al. 1999). Eventually, BBDR models for alterations in signaling motifs and nonlinear toxicological responses may be linked to organ system descriptions of physiology to predict both early responses (i.e., activation or deactivation of biological switches regulating signaling motifs) and adverse responses (i.e., diminished physiological function or diminished adaptability to stress). Such models would fulfill the goals proposed by Noble of having tools to simulate expected results, aid in experimental design, and predict biological/toxicological consequences throughout the full range of exposure situations with environmental toxicants (Noble 2002).

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