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Dynamic modeling of estrogen signaling and cell fate in breast cancer cells

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Abstract

Cancers of the breast and other tissues arise from aberrant decision-making by cells regarding their survival or death, proliferation or quiescence, damage repair or bypass. These decisions are made by molecular signaling networks that process information from outside and from within the breast cancer cell and initiate responses that determine its survival and reproduction. Because the molecular logic of these circuits is difficult to comprehend by intuitive reasoning alone, we present some preliminary mathematical models of the basic decision circuits in breast cancer cells, with an eye to understanding better their susceptibility or resistance to endocrine therapy.

Cancer is a collection of diseases characterized by misregulation of the biomolecular pathways that control cellular processes of metabolism and growth, DNA replication and repair, mitosis and cell division, autophagy and apoptosis (programmed cell death), de-differentiation, motility and angiogenesis¹. Molecular cell biologists have amassed a large body of information about the genes and proteins involved in these pathways and have some good ideas about how they go awry in certain types of cancers. However, most of our understanding of the molecular basis of cancer relies on intuitive reasoning about highly complex networks of biochemical interactions^{2–4}. Intuition is clearly not the most reliable tool for querying the behavior of complex regulatory networks. Wouldn't it be better if we could frame a reaction network in precise mathematical terms and use computer simulations to work out the implications of how the network functions in normal cells and malfunctions in cancer cells?

Of primary interest to cancer biologists is how cancer cells differ from normal cells in their responses to endogenous signals (such as growth and death factors, cell-cell and cell-matrix

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SUPPLEMENTARY INFORMATION

S1 (box) | S2 (box) | S3 (box) | S4 (box) | S5 (box)

contacts) and to exogenous treatments (including cytotoxins, radiation, endocrine therapy). Cell responses—signal transduction, cell-fate decisions, adaptation—are intrinsically dynamic phenomena, so it is essential to understand the temporal evolution of biochemical signaling networks in response to particular stimuli. Ordinary differential equations, based on biochemical reaction kinetics, are an appropriate tool for addressing these questions. In principle, ODE models can provide a comprehensive, unified account of many experimental results, and a reliable tool for predicting novel cell behaviors. ODE models of yeast cell growth and division have lived up to these expectations^{5–8}. But is it possible to build useful models of the considerably more complex regulatory networks in mammalian cells? We intend, in this article, to provide a roadmap for a detailed mathematical model of the estrogen signaling network in breast epithelial cells.

Our roadmap is built on the idea that a cell is an information processing system, receiving signals from its environment and its own internal state, interpreting these signals, and making appropriate cell-fate decisions, such as growth and division, movement, differentiation, self-replication, or cell death⁹. In plants and animals, these cell-level decisions are crucial to the growth, development, survival and reproduction of the organism. A hallmark of cancer cells is faulty decision-making: they proliferate when they should be quiescent, they survive when they should die, they move around when they should stay put¹. To understand the origin, pathology and vulnerabilities of cancer cells, we must understand how normal cells make decisions that promote the survival of the organism as a whole, and how cancer cells make decisions that promote their own survival and reproduction with fatal results for the organism they inhabit¹⁰.

Viewing the living cell as an information processing system, we can (conceptually, at least) distinguish an input level, a processing core, and output devices (FIG. 1). As input, a cell receives information from its surroundings (such as extracellular ligands that bind to cell-surface receptors or to nuclear hormone receptors) and from its internal state (such as DNA damage, misfolded proteins, low energy level and oxidative stress). These signals are processed by chemical reaction networks that integrate information from many sources and compute a response. A response could take the form of the activation or inactivation of key integrator or effector proteins that drive the cell's functional output devices. Of most interest to cancer biologists are the functional modules that control cell growth and division, motility and invasion, stress responses and apoptosis.

Although there may be many ways to subdivide the information processing system of a cell, there is clearly a need to divide and conquer the staggering complexity of the system^{11–13}. Fortunately, it is not necessary to model the protein reaction networks in all their complexity, because it is usually possible to identify a set of key 'integrator' and 'decision-making' proteins that determine the cell's response to input signals. Unfortunately, living cells are not like human-engineered systems, where modules are designed not to interfere much with one another¹⁴. Cellular modules have significant crosstalk and shared components. So although we must divide the system into modules to reduce the initial modeling complexity, we must also put the modules back together into a complete system that properly captures the information processing capabilities of living cells.

A comprehensive model of the information processing system of mammalian cells is not yet available, but we can provide a roadmap of how a modeler might capture, in mathematical form, the molecular events controlling cell growth, proliferation, damage responses and programmed death. Our approach is illustrated by simple mathematical models of the mechanisms involved in the initial susceptibility of breast cancer cells to anti-estrogen therapy and their subsequent development of anti-estrogen resistance. The value of this enterprise will be measured ultimately by new insights provided by the model into the logic

and functionality of estrogen-receptor signaling pathways and by the effectiveness of the model as a tool for experimental prediction and design.

The estrogen receptor and breast cancer

The growth and proliferation of breast tissue is normally responsive to estrogen, a steroid hormone that binds to and activates the estrogen receptors (ER α and ER β), nuclear transcription factors that regulate the expression of genes that orchestrate survival and proliferation. In many neoplastic breast cells, the ER signaling network contributes to controlling the relative rates of cell proliferation and programmed cell death, with pro-survival and proliferation signals overwhelming pro-death and quiescence signals.

Of the 180,000 cases of invasive breast cancer newly diagnosed each year in the USA, more than 70% express ER α (ER⁺ cells)¹⁵. Many of these tumors are initially responsive to endocrine therapy alone, and many also respond to a combination of cytotoxic chemotherapies^{16,17}. Endocrine therapy can consist of anti-estrogens (such as Tamoxifen or Fulvestrant), which bind to and neutralize ER, and/or aromatase inhibitors (such as Letrozole or Exemestane), which block the synthesis of estrogen. Unfortunately, many ER⁺ tumors recur as incurable, endocrine-resistant cancer cells¹⁸.

The advantages and limitations of endocrine therapies have been known for over 30 years. To make significant new advances in the treatment of advanced breast cancer, we need a better understanding of the ER signaling network¹⁹. For example, how does ER signaling function in normal breast cells? How does it malfunction in ER⁺ breast cancer cells that respond to endocrine therapy? How is it further misregulated in antiestrogen-resistant and aromatase inhibitor-resistant cancer cells? And how are cell survival and proliferation maintained in ER cancer cells?

FIGURE 1 provides an overview of the ER signaling network and its major output devices (cell growth and division, apoptosis and autophagy). From a combination of classical molecular biology studies and high-throughput transcriptomic analyses, we identified an initial set of transcription factors that are intimately connected with ER signaling in breast cancer cell lines²⁰. Subsequently, we and others have established the functional relevance of several of these factors, including NF κ B, a pro-survival transcription factor that is highly expressed in hormone-resistant cells compared to hormone-sensitive cells^{21–23}; IRF1, a pro-death transcription factor that is down-regulated in endocrine-resistant cells^{24–27}; and XBP1, a transcription factor involved in the unfolded protein response and induction of autophagy, which is highly expressed in its active (spliced) variant in endocrine-resistant cells^{27,28}. Given that FIG. 1 correctly captures some of the key regulatory components and their interactions, interpreting it at a mathematical level should provide novel and useful insights into the decision-making processes in normal and transformed breast cells.

Mathematical modeling perspective

As useful as FIG. 1 is for providing a guide to intuitive reasoning about the probable effects of perturbations to this network, a molecular interaction graph can deliver much more information about the potential dynamic behavior of the control system if it is translated into reasonable mathematical terms suitable for computer simulation. In that case, the computer can keep track of the dynamic consequences of multiple and often conflicting interactions^{29,30}.

In keeping with our roadmap perspective, we will begin by modeling the separate modules in FIG. 1: the ‘decision modules’ (cell cycle and apoptosis), the ‘stress modules’ (autophagy and unfolded protein response), and the ‘signal processing modules’ (ER and growth factor

signal transduction networks). As we go along, we will describe how the ‘integrator and effector proteins’ mediate communication between these modules.

Cell cycle module

We start with the module controlling DNA replication and division, events that are triggered by cyclins and cyclin-dependent kinases (CDKs)^{31,32}, the retinoblastoma protein (RB), which regulates members of the E2F-family of transcription factors, and late-G1 and early-S phase cyclins (type A and E cyclins)^{33–35}. RB also down-regulates the expression of ribosomal RNA genes, thereby inhibiting the production of new ribosomes and the cell’s capacity for increased protein synthesis^{34,36–39}. Hence, we can think of RB as a major ‘brake’ on cell growth and division, which must be released before a cell can grow and divide. This release is the job of the cyclin D-dependent kinases (CYCD1–3 in combination with CDK4 or CDK6), which phosphorylate RB and reduce its inhibitory effect on E2Fs^{33,40}. CYCD is an unstable protein, and it is not present in quiescent cells because its transcription regulators, including MYC, AP1 and β -catenin, are inactive. These transcription regulators are activated by proliferative signals, such as growth factors, cytokines, nuclear hormone receptors and integrins, causing the concentration of CYCD to rise. The increasing concentration of CYCD must be converted into a digital decision: shall the cell undergo a new round of DNA replication and division or remain in G1 phase?

This decision is apparently made by a bistable switch, created by the interaction between RB, E2F and cyclin E (CYCE)^{41,42}. The molecular interactions among these three proteins (FIG. 2a) are characterized by a positive feedback loop (E2F up-regulates CYCE, CYCE–CDK2 inactivates RB, RB inactivates E2F) and an auto-activation loop (E2F family members can activate their own transcription). According to mathematical models (Ref. 41 and supplementary information S1 (Box)), these sorts of positive feedback loops create a signal-response curve (FIG. 2b) with alternative stable steady states: an OFF state (RB active, E2F low, CYCE low), and an ON state (RB inactive, E2F high, CYCE high). The OFF state corresponds to quiescent cells (arrested in G1 phase of the cell cycle) and the ON state to proliferating cells (progression through S-G2-M phases)⁴³. Careful measurements of the expression of CYCD and E2F in fibroblast cells responding to changes in serum concentration confirm the predictions of the model (FIG. 2c and d)⁴¹. Entry into the mammalian cell cycle in these non-cancerous cells is controlled by a bistable switch that is biased to the OFF state by signals that down-regulate CYCD and E2F (and possibly by signals that up-regulate RB), and that is switched ON by signals that up-regulate CYCD and E2F (see supplementary information S1 (Box) for further information). In cancer cells, this crucial decision point may still be intact⁴⁴, but it is likely that in many cancers the bistable switch is disrupted by mutations that break the underlying feedback circuits⁴⁵.

CYCD as a key signal integrator

CYCD is a classic integrator and effector protein: its level integrates the pro- and anti-proliferative signals being received by the cell, and the activity of CYCD-dependent kinases effects the commitment of the cell to a new round of DNA synthesis and cell division. Pro-proliferative signals, such as estrogen acting through ER α , increase *CYCD* expression by activating its transcription factors. By contrast, cell-cell contacts result in cytoplasmic sequestration of β -catenin and down-regulation of *CYCD* expression. One of the hallmarks of many cancers is the loss of contact inhibition. A different mode of action is exemplified by the anti-proliferation factor, transforming growth factor β (TGF- β), which up-regulates synthesis of p27^{Kip1}, an inhibitor of CYCD-dependent kinases. In breast cancer cells TGF- β is a key regulator of the anti-proliferative effects of anti-estrogens^{46,47}, and *CYCD* expression is associated with poor response to Tamoxifen⁴⁸. In summary, we might think of CYCD level as a rheostat that varies up and down continuously in response to pro- and anti-

proliferative signals, respectively^{41, 43}. When CYCD level exceeds a certain threshold, the RB-E2F-CYCE switch converts the CYCD signal into a discrete decision to begin a new round of DNA synthesis and cell division. Triggering this switch is therefore dependent on many factors affecting the level of active CYCD, such as estrogen, β -catenin, p27^{Kip1} and TGF- β ^{49,50}.

Once a cell has committed to the G1/S transition, it will proceed through S, G2 and M phases, even if the pro-proliferative signals are removed and CYCD disappears. However, when this cell divides and the other classes of cyclins (A, B and E) are degraded, RB will return and arrest the cell in a quiescent state.

Apoptosis module

Like the decision to enter a new round of mitotic cell division, the commitment to apoptosis must reach an all-or-none decision point that is biased one way or the other by the summation of pro-death and pro-survival signals. Although the evidence is not conclusive, we believe that the irrevocable commitment to apoptosis is normally made in the activation of BAX and amplified by mitochondrial outer-membrane permeabilization (MOMP)⁵¹. In our mathematical models MOMP is governed by a bistable switch involving three families of proteins: BCL2-like, BH3-only and BAX-like proteins (FIG. 3a)^{52–57}. In the OFF state, BAX is inactivated by binding to BCL2. Accumulation of BH3 proteins can displace BCL2 from BAX, leading to the self-amplifying activation of BAX (the ON state). Active BAX proteins create pores in the mitochondrial outer membrane, thereby releasing cytochrome C and SMAC to the cytoplasm, where cytochrome C promotes activation of ‘executioner’ caspases, and SMAC neutralizes the inhibitor of apoptosis (IAP) proteins that inhibit caspases⁵⁵.

Based on our models (FIG. 3 and supplementary information S2 (Box)), the apoptosis-switch is in the OFF or ON position depending on the balance between BCL2-like proteins (the ‘brakes’) and BH3-only proteins (the ‘accelerators’). When the ratio of accelerators to brakes exceeds a certain critical value (the point in FIG. 3b where the OFF state disappears), then BAX is abruptly activated and MOMP-induced activation of executioner caspases ensues. The ‘snap-action’ kinetics of MOMP are consistent with this view of a bistable switch activating BAX proteins (FIG. 3c).

Whether or not apoptosis is controlled by a bistable ‘decision’ module and (if so) what is its biochemical basis are questions of considerable discussion in the literature^{51,53–56,58–62}. This debate illustrates that fundamentally different mathematical models may be equally consistent with limited experimental data. Fortunately, the different models can be used to design additional experiments that will distinguish between alternative mechanisms. We suppose that apoptosis is governed by a one-way (irreversible) bistable switch, because apoptosis in normal cells is an all-or-nothing affair. We interpret the evidence to suggest that the decision is made upstream of MOMP and that the BH3-BCL-BAX module is the most likely locus for the bistable switch. Although the apoptotic switch may be disabled in some cancer cells, it is likely still functional in most cancers but more difficult to engage. For instance, in breast cancer cell lines, ER-mediated signaling upregulates antiapoptotic proteins, including BCL2^{23,63,64}, BCL-W⁶⁴ and BCL3⁶⁵, making it harder to trigger apoptosis. Endocrine-therapy, by inactivating ER, moves these levels in the opposite direction, making it easier to trigger apoptosis.

Damage-processing modules

Intracellular damage-processing modules have crucial roles in maintaining the viability of cells and organisms. For example, DNA damage activates kinases that phosphorylate and

stabilize the transcription factor p53 (Ref.⁶⁶). p53 up-regulates genes encoding repair enzymes and p21^{Cip1}, which binds to and inhibits the activity of CDKs, thereby preventing the damaged cell from beginning a new round of DNA replication. DNA damage also prevents S- or G2-phase cells from entering mitosis by pathways involving inhibitory phosphorylation of CDKs and the production of stoichiometric CDK inhibitors. If the damage cannot be repaired in a timely fashion, then p53 upregulates production of BH3 proteins in an attempt to activate the apoptosis module. Whether apoptosis occurs or not depends on BH3 level relative to BCL proteins, thereby integrating the influences of pro- and anti-apoptotic agents, including ER-mediated signals. Effective mathematical models of these DNA damage-processing pathways, based on the cell-proliferation and cell-death networks described in FIG. 2 and 3, have been published^{52,55,66,67}.

Other common inducers of stress in normal and cancer cells include hypoxia and oxidative stress^{68,69}. These types of stress cause problems in intermediary metabolism, electron transport in mitochondria and protein folding in the endoplasmic reticulum, and these problems induce characteristic responses by the cell. The first response to low-level stress is a pro-survival mechanism, autophagy, which is thought to provide a steady supply of energy and raw materials by degrading the cell's own proteins and lipids⁷⁰. Unremitting stress can lead to cell death, either by excessive autophagy or by activation of apoptosis⁷¹.

Autophagy module

The autophagosome is a subcellular organelle containing a selection of cellular proteins and other macromolecules that are destined for destruction. When the autophagosome fuses with a lysosome, its contents are hydrolyzed to amino acids and other small metabolites that can be used by the cell as sources of energy and raw materials for the biosynthesis of essential substances. Autophagy is controlled in large part by Beclin-1 (BECN1), a myosin-like, BCL2-interacting protein. When not bound to BCL2, BECN1 participates in a multi-protein complex that initiates the earliest stages of autophagosome assembly⁷⁰⁻⁷².

In FIG. 4 we propose a simple model for the initiation of autophagy (for details, see supplementary information S3 (Box). In this model, autophagy is regulated not as a toggle switch (as in FIG. 2 and 3) but as a rheostat, ramping up smoothly to higher levels as stress increases. As autophagy ramps up, BCL2 is released from its association with BECN1 and with the inositol trisphosphate receptor (IP₃R). The results can be quite variable, including survival (moderate autophagy and inhibition of apoptosis), or apoptotic cell death, or autophagic cell death. Whether the autophagic response is functioning normally or abnormally in breast cancer cell lines is a matter of current investigation.

The unfolded protein response (UPR) module

The accumulation of unfolded proteins in the endoplasmic reticulum causes a characteristic response⁷³ that is intended to relieve the immediate problem (by re-folding or degrading the non-functional proteins and reducing the rate of protein synthesis) and to deal with the underlying stress (by inducing autophagy). The molecular basis of the UPR is well understood, and useful mathematical models have been presented in the literature⁷³⁻⁷⁶. In FIG. 5 and supplementary information S4 (Box) we present a simplified model of UPR, to illustrate the basic principles of this damage-response module. Both autophagy and the UPR are strongly implicated in the responsiveness of breast cancer cells to anti-estrogens^{19, 77}.

Signaling crosstalk between modules

To impose some order on the tangled web of macromolecular interactions within a living cell it is necessary to think in terms of functional modules. Nonetheless, we must take into account that there is significant crosstalk between modules; for example, the apparent

mutual inhibition between autophagy and apoptosis⁷¹. Crosstalk between signal transduction pathways is well known; for example, overlapping cell survival pathways are implicated in the notorious plasticity of cells in response to cancer chemotherapeutics, including endocrine therapies^{49,78–80}. Understanding the mechanisms and roles of crosstalk is a crucial concern as we try to assemble modules into more complex networks that can account for the complex responses of cells under realistic conditions, including the development of drug resistance in breast cancer cells.

As an example, consider the epidermal growth factor (EGF) family of signaling pathways. A growing body of evidence demonstrates that endocrine therapy, which is often effective in regression of early-stage ER⁺ breast cancer, may provoke cellular adaptation processes including activation of a spectrum of estrogen-regulated survival and proliferation genes, such as those involved in EGF signaling^{81–86}. Interestingly, Pratt et al.⁶⁵ observed that a population of MCF-7 cells could be divided into two sub-groups following withdrawal of estrogen: most cells retain an absolute dependency on estrogen and die as a result of the treatment, but some cells become estrogen-independent by switching to alternative survival and proliferation signals. If endocrine treatment is discontinued within a short period of time, before the resistant cells have established their phenotype by genetic or epigenetic modifications, then the acquired resistance can be reversed. For example, a population of MCF-7 cells that over-express EGFR or HER2 exhibit a bimodal distribution of receptors (FIG. 6c), and this distribution pattern can be reversibly controlled by manipulating the exposure of the cells to estrogen^{87–89}. We take these observations as evidence for a bistable survival-switch working through crosstalk between ER and EGF signaling pathways. Although little is known about how it works, mutual inhibition between the two pathways is a likely source of bistability. In FIG. 6 and supplementary information S5 (Box) we present a simple model that could account for the effects of estrogen withdrawal on MCF-7 cells.

Crosstalk in cell signaling networks generates a large selection of discrete, stable, self-organized states; creating a degree of cell-fate plasticity that is necessary for a cell to switch adaptively and robustly among these different states. Although this plasticity is essential for normal cells to survive in noisy environments and to differentiate properly in response to various developmental cues, it may lead to robust development of resistance to cytotoxic drugs. Hence, understanding how crosstalk controls these phenotypic switches is of first importance for designing more effective cancer treatment strategies.

Present Realities and Future Directions

Mathematical modeling of intracellular molecular regulatory networks is an essential part of a systems-approach to cancer biology⁹⁰. Intuitive reasoning must be complemented by mathematical models when the molecular regulatory network under consideration is large, complex and interconnected, and when we are dealing with quantitative aspects of signaling and control⁹¹. A well crafted mathematical model allows us to integrate crucial information about the genetics, molecular biology and physiology of cancer cells into a quantitative hypothesis, amenable to computer simulation, mathematical analysis, and detailed comparison to experimental data. By computing the behavior of the model under a variety of experimental conditions and comparing these simulations to the observed behavior of cells, we can determine whether our hypothetical molecular mechanism is sufficient to account for the known behavior of cells. If and when our model passes this first test ('post-diction'), we can use it to predict the behavior of cells under novel experimental conditions, and use these quantitative predictions to test the efficacy of the model. Even when are models are not in full agreement with experiments, we can be confident that the problem is in some part of the model rather than in faulty reasoning about its consequences. Indeed, the model can help us track down the origin of the problem(s) and consider alternative hypotheses.

Mathematical modeling of intercellular control systems related to breast cancer development, though still in its infancy, is beginning to provide some useful insights. For example, a sophisticated model of p53 signaling in MCF-7 cells successfully predicted a novel role for Wip1 in a negative feedback loop from p53 to an upstream kinase in the DNA damage-signaling pathway⁹². A recent model of the HER2-ER signaling network identified novel drug targets for trastuzumab-resistant cells⁹³. A dynamic model of combinatorial cancer therapy suggested promising treatment strategies that were subsequently verified experimentally⁹⁴.

In this review, we have presented a roadmap for the mathematical modeling component of an integrative, systems biology of endocrine responsiveness in ER⁺ breast cancer. The hard work is yet to be done: formulating and verifying models, estimating kinetic parameters, making non-obvious predictions and testing them by quantitative experimental measurements. Is it just a matter of time before an effective, integrated model of regulatory networks in breast cancer cells is informing the next wave of experiments and therapies? Successful ODE models of cell cycle regulation, growth factor signaling, programmed cell death and the unfolded protein response suggest that there are no fundamental barriers to accurate, predictive models of complex control systems in mammalian cells. On the other hand, effective modeling is hampered by many significant differences—genetically and phenotypically—among different types of mammalian cells. Extending models to cancer cells, which are notoriously unstable genetically, will be even more difficult. High-throughput data collection and analysis will be helpful in identifying important differences among cell types and between normal cells and their cancerous derivatives^{95–97}.

Despite the seeming wealth of data on molecular mechanisms controlling mammalian cell proliferation and stress responses, there is often a distinct lack of reliable, quantitative measurements of these mechanisms under conditions that are relevant to model formulation and testing. Another impediment to modeling intracellular control systems stems from the fact that the behavior of populations of cells (e.g., graded response to drug treatment) may not reflect the behavior of single cells (all-or-none decision in response to the drug). At present, modelers are still struggling with how best to cope with all these competing issues.

In addition, there are other relevant theoretical considerations that we have not described in this review. For instance, realistic models of molecular regulation must take into account the compartmentalization of eukaryotic cells. For another, the restricted number of genes, mRNAs and protein molecules in a single cell generate unavoidable stochastic fluctuations in molecular control networks. Intracellular information-processing systems must be robust to these fluctuations in most circumstances, although in some circumstances these fluctuations may be exploited to generate a range of possible outcomes ('bet-hedging'). Thirdly, our models only bridge the scales from molecular networks to cell physiology. Breast tumors exist in a complex microenvironment that affects the dynamic signaling within and among cancer cells. Modeling these effects adds new layers of complexity. Other kinds of mathematical models are needed to describe how cells are organized into multicellular tissues, interacting with extracellular matrix, recruiting vasculature, and eventually metastasizing to distant parts of the body^{98–100}. Models at these higher scales are beginning to be integrated with molecular-level descriptions of intracellular control systems (e.g., the cell cycle) and of intercellular communication (e.g., Wnt signaling)^{101,102}.

We expect that all these modeling challenges will soon be overcome and that a new generation of mathematical models will soon be providing new insights into the molecular foundations of endocrine responsiveness in breast cancer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Glossary

Module	A set of molecular interactions that accomplishes a specific task in a cell, such as committing a cell to a new round of DNA replication
Crosstalk	Interactions among modules that alter the behavior of the modules in isolation
Unfolded protein response	Cellular response to the accumulation of stress-induced, misfolded proteins in the endoplasmic reticulum. Cells down-regulate general protein synthesis and up-regulate production of chaperones that help damaged proteins to re-fold properly or to be degraded
Autophagy	Degradation of a cell's own components, using its lysosomal machinery, to remove damaged organelles and/or to provide energy and raw materials for adaptation and survival under stressful conditions
Molecular interaction graph (wiring diagram)	Representation of a set of biochemical reactions involving co-regulated genes and proteins; for example, a signal transduction pathway or a transcription factor network
Dynamic behavior	The characteristic change over time of a molecular regulatory network in response to a specific pattern of input signals
Bistable switch	A regulatory network that can persist, under identical external conditions, in either of two stable states ('on' or 'off') depending on its recent history
Signal-response curve	The functional dependence of the output of a molecular regulatory network (e.g., the activity of a transcription factor) on changing values of its input (e.g., concentration of a growth factor in the extracellular medium)
Rheostat	A variable resistor, used to provide continuous control over the current through a circuit (e.g., the dimmer knob on a light fixture)
Endocrine therapy	Manipulation of endocrine levels or responsiveness to prevent the survival and proliferation of hormone-dependent cancer cells
Plasticity	The ability of a regulatory network, in the face of interference or damage, to adapt and maintain something akin to its normal function
Stochastic fluctuations	Random variations in the numbers of molecules of mRNAs and proteins due to the unpredictable nature of chemical reactions at the molecular level

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FIGURE 1. The estrogen receptor signaling network in breast epithelial cells

Extracellular signals, such as estrogen (E2), growth factors (GF), survival factors (SF), cytokines (cyto) and extracellular matrix (ECM), bind to receptor proteins, which initiate a complex series of chemical reactions within the cell, culminating ultimately in the activation of a set of integrator/effector proteins. These proteins sum up the positive and negative signals coming from the information processing units, and then they drive responses in the downstream decision modules and stress modules. The ‘cell cycle module’ coordinates DNA synthesis and mitotic cell division with cell growth and the body’s need for a continuous supply of new cells in the right place at the right time. The ‘apoptosis module’ rids the body of damaged, worn out, or un-needed cells. The ‘unfolded protein module’ is a response to stresses such as starvation and reactive oxygen species. Under conditions of extreme stress, the ‘autophagy module’ can provide the cell with a supply of energy and raw materials. Tamoxifen (Tam) and Faslodex (Fas) are inhibitors of the estrogen receptor (ER α), and they are commonly used to kill estrogen-dependent breast cancer cells.

FIGURE 2. Bistable switch controlling the G1-to-S transition in mammalian cells

a | Wiring diagram. Cyclin E-dependent kinase (CycE) promotes the transition of mammalian cells from G1 phase of the cell cycle into S phase. Quiescent cells are arrested in G1 by the retinoblastoma protein (RB), which binds to and inhibits E2F, a family of transcription factors, some of which can promote *CYCE* gene expression. Phosphorylation of RB by cyclin-dependent kinases compromises its inhibitory effect on E2Fs. The initial phosphorylation of RB is accomplished by cyclin D-dependent kinase (CYCD). After the G1/S transition is made, RB is maintained in its inactive (phosphorylated) form by CYCE and by cyclin A- and B-dependent kinases that are active in S-G2-M. Quiescent cells, which have very little CYCD, can be induced to proliferate by transcription factors (such as Myc, Fos, Jun) that are upregulated by growth factors in serum. These transcription factors promote the expression of both *CYCD* and *E2F* genes, and E2F proteins upregulate their own transcription. **b** | Signal-response curve. The wiring diagram in panel a is converted into a set of nonlinear differential equations (supplementary information S1 (Box)), and the steady-state levels of CYCD and E2F are plotted as functions of serum concentration in the growth medium. Although CYCD level increases smoothly with serum concentration ('rheostat'), the E2F distribution exhibits a bimodal dependence on serum concentration ('toggle switch')^{41,43}. **c** and **d** | Experimental verification in rat embryonic fibroblasts (REF 52 cells); from Yao et al.⁴¹, used by permission. Red curves: serum concentration is raised from 0 to final percentage. Green curves: serum concentration is raised to 20% for 5 hours, then lowered to final percentage. GFP fluorescence is driven by an E2F promoter (left) or a CYCD promoter (right). Histograms of CYCD expression shift smoothly up-and-down with final serum percentage. E2F histograms show a bimodal dependence on final serum percentage between ~0.2% and 2%. Notice a distinct hysteresis effect in the E2F response: on shifting serum up (red curves), bistability is not observed until serum level exceeds ~1%, but on shifting serum down (green curves), bistability is maintained to serum levels < 0.2%.

FIGURE 3. Bistable switch controlling apoptosis in mammalian cells

a | Wiring diagram. Programmed cell death is triggered by activation of BAX proteins in the outer membrane of mitochondria. Active BAX causes the membrane to become permeable to proteins, such as cytochrome C and SMAC, which induce the activation of proteases (caspases) and other hydrolytic enzymes that disassemble the macromolecules of the cell. BAX is activated by BH3-family proteins and kept inactive by binding to BCL-family proteins. BH3 proteins also bind to BCL proteins⁵⁵. **b** | Signal-response curve. A mathematical model of the wiring diagram is presented in supplementary information S2 (Box). Top: steady-state concentration (black curve) of total membrane-bound BAX, $[BAXm]^T = [BAXm] + [BAXm:BCL2]$, as a function of $[Stress]$. For intermediate levels of Stress, the network has two stable steady states: an OFF state with low total level of BAXm, all of it complexed to BCL2 (i.e., $[BAXm] \approx 0$); and an ON state with high total level of BAXm, most of it un-complexed to BCL2 (i.e., $[BAXm] \approx [BAXm]^T$). Bottom: time-course of active BAX. Each simulation is started in the naïve state: no stress, low level of BH3, $[BAXm] \approx 0$. At $t = 0$, $[Stress]$ is raised to a final value that varies (from one simulation to the next) from 0.55 to 0.95. The time-course for final $[Stress] = 0.95$ is plotted as the blue curve on the upper graph. Notice that, in each simulation, there is a long lag time followed by an abrupt activation of BAX (and subsequently an irreversible activation of caspases). The duration of the lag phase is a decreasing function of $[Stress]$. **c** | Experimental verification in HeLa cells (cervical cancer cell line); from Albeck et al.⁵¹, used by permission. Top: Cells are treated with 50 ng/ml TRAIL (TNF-related apoptosis-inducing ligand) and assayed for caspase activity by cleavage of an artificial substrate manufactured by the cell. Cells with high caspase activity are pseudo-colored yellow. Notice the long lag time before caspases are activated in any cells, then individual cells activate caspases abruptly, but there is a wide dispersion of activation times among cells. Bottom: traces of caspase activity in single cells activated by different concentrations of TRAIL.

FIGURE 4. The interplay between autophagy and apoptosis

a | Wiring diagram. In response to stress, both Beclin-1 and BCL2 are phosphorylated, causing the BCL2:Beclin-1 complex to dissociate^{103,104}. Beclin-1 is phosphorylated by DAPK (death-associated protein kinase), and BCL2 is phosphorylated by JNK, a downstream target of the IRE1 arm of the UPR^{75,103,104}. Detachment from the extracellular matrix provides an additional stress to the cells¹⁰⁵. Free Beclin-1 participates (with other components, such as Atg8/LC3) in initiating autophagy⁷¹. Autophagy can suppress the stress signal by providing the cell with ATP and raw materials for new protein synthesis. BCL2 phosphorylation also allows the inositol trisphosphate receptor (IP₃R) to release calcium from the endoplasmic reticulum to the cytoplasm^{103,106}. If the concentration of calcium in the cytoplasm gets large enough, apoptosis is triggered¹⁰⁶. Activated caspases cleave Beclin-1 and turn off autophagy¹⁰⁷. Hence, under low stress conditions, autophagy promotes cell survival; at moderate stress, it may lead to autophagic cell death; and for large stress, calcium release may stimulate apoptosis by the intrinsic (mitochondrial) pathway. **b |** Numerical simulations. The wiring diagram is converted into a set of ordinary differential equations (supplementary information S3 (Box)), and the fraction of cells predicted to stain positive for autophagic vesicles is plotted as a function of time. 1) Detached and high stress; 2) attached and moderate stress; 3) detached and no stress. **c, d |** Experimental evidence in MCF-7 cells (breast cancer cell line); from Petrovski et al.¹⁰⁵, used by permission. Panel **c**: Percentage of cells that have taken up monodansylcadaverin (MDC) into autophagic vacuoles on days 1–7 after the following treatments: (▽) 10% fetal calf serum (FCS) on poly-HEMA coated plates; non-adherent cells undergo autophagy by day 6 and later die by apoptosis (not shown). (▼) 3% charcoal-stripped FCS on poly-HEMA plates; estrogen-depleted, non-adherent cells undergo autophagy by day 4 and later die by apoptosis. (○) 3% charcoal-stripped FCS + Tamoxifen; estrogen-depleted, ER-inhibited cells undergo autophagy by day 4. (□) 3% charcoal-stripped FCS + Tamoxifen on poly-HEMA plates; estrogen-depleted, ER-inhibited, non-adherent cells undergo autophagy by day 2 and later die by apoptosis. Panel **d** (top): Transmission electron microscopy of (1) untreated, control cells and of (2) autophagic cells (○) on day 4, showing numerous autophagic vacuoles (white) in the cytoplasm. Panel **d** (bottom): Western blot analysis of LC3 expression in autophagic cells (○) on days 0–7; c = positive control.

FIGURE 5. The unfolded protein response in mammalian cells

a | Wiring diagram. The unfolded protein response (UPR) is a coordinated cellular program that is induced by the accumulation of unfolded and misfolded proteins in the lumen of the endoplasmic reticulum⁷³. Elevated levels of unfolded/misfolded proteins are brought down by chaperones, foldases, oxidoreductases, glycosylases and proteases (we refer to all these components simply as chaperones). As unfolded proteins pull chaperones away from the luminal domains of PERK, ATF6, and IRE1, then these three proteins up-regulate the expression of certain genes that reduce the stress and increase the protein folding capacity of the endoplasmic reticulum. TA = translation attenuation. **b** | Numerical simulations computed from the mathematical model in supplementary information S4 (Box). Top and middle: at $t = 0$, with the model at a stable resting state, a stress of 10 (arbitrary units) is added to the differential equations, and the response of the system is plotted in terms of total unfolded protein, total chaperone, and protein species not bound to chaperones (UP, IRE1_{active} and PERK_{active}). Bottom: response to a stress of 5 a.u. applied at $t = 0$. **c** | Experimental verification in non-cancerous cells. Top: Time courses of the three stress sensors after treatment of CHO (chinese hamster ovary) cells with 10 $\mu\text{g/mL}$ of tunicamycin (Tm) to induce protein misfolding; from DuRose et al.¹⁰⁸, used by permission. Bottom: Time course of IRE1 activity (assayed as % splicing of XBP1 mRNA) in HEK293 (human embryonic kidney) cells treated with 5 $\mu\text{g/mL}$ of tunicamycin; from Li et al.¹⁰⁹, used by permission.

FIGURE 6. Crosstalk between ER and EGF signaling pathways

a | Wiring diagram. E2 = estrogen, ER = estrogen receptor alpha, GF = growth factors, GFR = growth factor receptors. GFR and its downstream signaling network are suppressed by ligand-dependent ER signaling ('ER classic') but promoted by ligand-independent ER signaling ('ER non-classic'). Increased GF signaling after E2 withdrawal can shift ER from classical to non-classical signaling. GF signaling can up-regulate its own activity. **b** | Signal-response curve. Differential equations describing the wiring diagram are provided in supplementary information S5 (Box). The steady-state activity of GFR is plotted as a function of [E2] in the growth medium (from 0 to 5 pM). At any given [E2] in this range, a cell may express a low or high level of GFR (upper and lower solid lines; the middle dotted line indicates a branch of unstable steady states). **c** | Experimental evidence; from Liu et al.⁸⁸, modified and used by permission. Bimodal distribution of HER2 (ErbB-2) expression in a monoclonal culture of HER2-overexpressing MCF-7 cells. The bimodal distribution pattern is induced by a change in [E2]. Cells grown on charcoal-stripped calf serum (CCS) for 5 weeks or longer are depleted of E2 and constitute a single population of high HER2-expressing cells by flow-cytometric analysis (top plot, abundance of HER2 as detected by fluorescent antibody). Replacing CCS with FCS (fetal calf serum, containing E2) leads to the emergence over time (5 weeks, 3 months, 4 months) of a second population of low HER2-expressing cells. Similar results have also been observed in EGFR (ErbB-1)-overexpressing MCF-7 cells⁸⁷. **d** | Model simulation. In supplementary information S5 (Box), noise terms are added to the differential equations to take stochastic effects into account. 2000 cells are simulated with [E2] = 0 (corresponding to CCS) for 3.3 weeks, which results in a single population of high HER2-expressing cells (top plot). Setting [E2] = 5 pM (corresponding to FCS) leads to a time-course of HER2 expression histograms similar to the experimental observations in panel c.