OPINION

Dynamic modelling of oestrogen signalling 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 signalling networks that process information from outside and from within the breast cancer cell and initiate responses that determine the cell's 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 that may aid our understanding of their susceptibility or resistance to endocrine therapy.

Cancer is a collection of diseases that are 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 angiogenesis[1]. 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 behaviour of complex regulatory networks. Would it not 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 contacts) and to exogenous treatments (including cytotoxic radiation and endocrine therapies). Cell responses — such as signal transduction, cell-fate decisions and adaptation — are intrinsically dynamic phenomena, so it is essential to understand the temporal evolution of biochemical signalling networks in response to particular stimuli. Ordinary differential equations (ODEs), which are 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 they are a reliable tool for predicting novel cell behaviours. 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 oestrogen signalling network in breast epithelial cells.

Our roadmap is built on the idea that a cell is an information processing system: it receives signals from its environment and its own internal state, interprets these signals and makes appropriate cell-fate decisions, such as growth and division, movement, differentiation, self-replication or cell death[9]. 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, and they move around when they should stay put[1]. 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 inhabit[10].

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, in which modules are designed not to interfere much with one another[14]. Cellular modules have considerable crosstalk and several shared components. So although we must divide the system into modules to reduce the initial modelling 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 modeller 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-oestrogen therapy and their subsequent development of anti-oestrogen resistance. The value of this enterprise will be measured ultimately by new insights provided by the model into the logic and functionality of oestrogen receptor (ER) signalling pathways and by the effectiveness of the model as a tool for experimental prediction and design.

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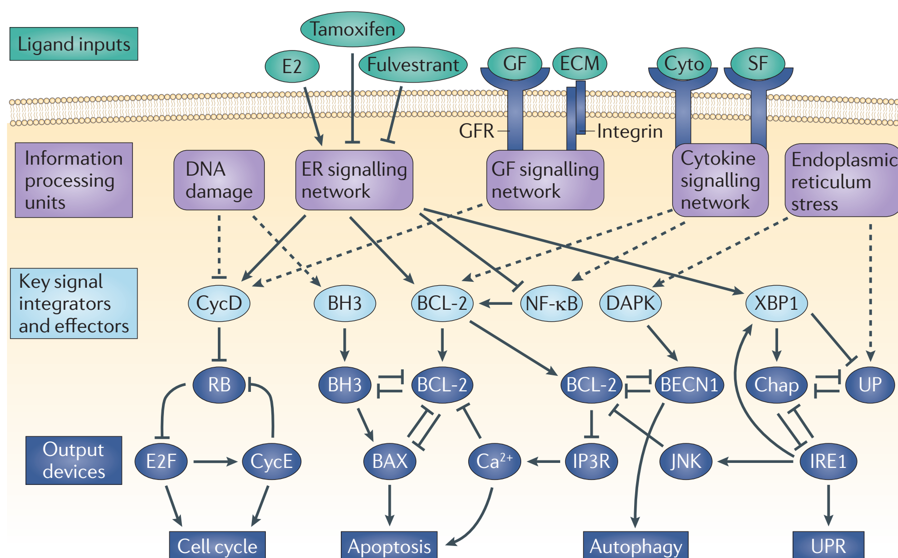

Figure 1 | **The oestrogen receptor signalling network in breast epithelial cells.** Extracellular signals, such as oestrogen (E2), growth factors (GF), survival factors (SF), cytokines (Cyto) and extracellular matrix (ECM), bind to receptor proteins, which initiate a complex series of chemical reactions within the cell, culminating ultimately in the activation of a set of integrator and effector proteins. These proteins process the positive and negative signals coming from the information processing units and then 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 unneeded cells. The 'unfolded protein response (UPR) 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 and fulvestrant are inhibitors of oestrogen receptor-α (ERα), and they are commonly used to kill oestrogen-dependent breast cancer cells. BECN1, beclin 1; Chap, chaperone; CycD, cyclin D; CycE, cyclin E; DAPK, death associated protein kinase; GFR, growth factor receptor; IP3R, inositol 1,4,5-triphosphate receptor; IRE1, inositol-requiring protein 1 (also known as ERN1); JNK, JUN N-terminal kinase; UP, unfolded protein; XBP1, X-box-binding protein 1.

The oestrogen receptor and breast cancer

The growth and proliferation of breast tissue is normally responsive to oestrogen — a steroid hormone that binds to and activates ERα and ERβ, which are nuclear transcription factors that regulate the expression of genes that orchestrate survival and proliferation. In many neoplastic breast cells, the ER signalling 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 United States, more than 70% express ERα (ER^+ cells)[15]. Many of these tumours 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-oestrogens (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 oestrogen. Unfortunately, many ER^+ tumours recur as incurable, endocrine-resistant cancer cells[18].

The advantages and limitations of endocrine therapies have been known for over 30 years. To make substantial new advances in the treatment of advanced breast cancer, we need a better understanding of the ER signalling network[19]. For example, how does ER signalling 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 anti-oestrogen-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 signalling 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 signalling in breast cancer cell lines[20]. Subsequently, we and others have established the functional relevance of several of these factors, including nuclear factor-κB (NF-κB), a pro-survival transcription factor that is highly expressed in hormone-resistant cells compared to hormone-sensitive cells[21–23]; interferon regulatory factor 1 (IRF1), a pro-death transcription factor that is downregulated in endocrine-resistant cells[24–27]; and X-box-binding protein 1 (XBP1), a transcription factor that is involved in the unfolded protein response (UPR) and the induction of autophagy and 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 epithelial cells.

Mathematical modelling 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 behaviour of the control system if it is translated into reasonable mathematical terms that are 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 modelling the separate modules in FIG. 1: the 'decision modules' (cell cycle and apoptosis), the 'stress modules' (autophagy and the UPR) and the 'signal processing modules' (ER and growth factor signal transduction networks). As we go, we will describe how the 'integrator and effector proteins' mediate communication among 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], RB (which regulates members of the E2F family of transcription factors (hereafter referred to collectively as E2F)) and late-G1- and early-S-phase cyclins (type A and E cyclins)[33–35]. RB also downregulates 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

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division that must be released before a cell can grow and divide. This release is the job of the cyclin D-dependent kinases (cyclin D1, cyclin D2 and cyclin D3 (hereafter collectively referred to as cyclin D) in combination with CDK4 or CDK6), which phosphorylate RB and reduce its inhibitory effect on E2F[33,40]. Cyclin D1 is an unstable protein, and it is not present in quiescent cells because its transcription regulators, including MYC, activator protein 1 (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 cyclin D to rise. The increasing concentration of cyclin D 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, which is created by the interaction among RB, E2F and cyclin E[41,42]. The molecular interactions among these three proteins (FIG. 2a) are characterized by a positive feedback loop (E2F upregulates cyclin E, cyclin E–CDK2 inactivates RB, and RB inactivates E2F) and an autoactivation loop (E2F family members can activate their own transcription). According to mathematical models (REF. 41 and Supplementary information S1 (text)), 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 and cyclin E low), and an ON state (RB inactive, E2F high and cyclin E high). The OFF state corresponds to quiescent cells (arrested in G1 phase of the cell cycle) and the ON state corresponds to proliferating cells (progression through S, G2 and M phases)[43]. Careful measurements of the expression of cyclin D and E2F in fibroblast cells responding to changes in serum concentration confirm the predictions of the model[41] (FIG. 2c,d). Entry into the mammalian cell cycle in these noncancerous cells is controlled by a bistable switch that is biased to the OFF state by signals that downregulate cyclin D and E2F (and possibly by signals that upregulate RB), and that is switched ON by signals that upregulate cyclin D and E2F (see Supplementary information S1 (text) for further information). Although this crucial decision point still seems to be intact[44] in many ER+ breast cancer cells, it is likely that in many cancers the bistable switch is disrupted by mutations that break the underlying feedback circuits[45].

Cyclin D as a key signal integrator. Cyclin D is a classic integrator and effector protein: its level integrates the proliferative and antiproliferative signals being received by the cell, and the activity of cyclin D-dependent kinases affects the commitment of the cell to a new round of DNA synthesis and cell division. Pro-proliferative signals, such as oestrogen acting through ERα, increase cyclin D expression by activating its transcription factors. By contrast, cell–cell contacts result in cytoplasmic sequestration of β-catenin and downregulation of cyclin D expression. One of the hallmarks of many cancers is the loss of contact inhibition. A different mode of action is exemplified by the antiproliferation factor transforming growth factor-β (TGFβ), which upregulates synthesis of p27 (also known as KIP1 and CDKN1B), an inhibitor of cyclin D-dependent kinases. In breast cancer cells, TGFβ is a key regulator of the antiproliferative effects of anti-oestrogens[46,47], and cyclin D gene expression is associated with poor response to tamoxifen[48]. In summary, we might think of cyclin D levels as a rheostat that varies up and down continuously in response to proliferative and antiproliferative signals, respectively[41,43]. When cyclin D levels exceed a certain threshold, the RB–E2F–cyclin E switch converts the cyclin D 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 that affect the level of active cyclin D, such as oestrogen, β-catenin, p27 and TGFβ[49,50].

After a cell has committed to the G1–S transition, it will proceed through the S, G2 and M phases, even if the proliferative signals are removed and cyclin D 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: BCL-2-like, BH3-only and BAX-like proteins[52–57] (FIG. 3a). In the OFF state, BAX is inactivated by binding to BCL-2. Accumulation of BH3 proteins can displace BCL-2 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 second

Glossary

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.

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.

Crosstalk
Interactions among modules that alter the behaviour of the modules in isolation.

Dynamic behaviour
The characteristic change over time of a molecular regulatory network in response to a specific pattern of input signals.

Modules
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.

Molecular interaction graph
A representation of a set of biochemical reactions involving co-regulated genes and proteins; for example, a signal transduction pathway or a transcription factor network. Also referred to as a 'wiring diagram'.

Plasticity
The ability of a regulatory network, in the face of interference or damage, to adapt and maintain something akin to its normal function.

Rheostat
A variable resistor, used to provide continuous control over the current through a circuit (for example, the dimmer knob on a light fixture).

Signal–response curve
The functional dependence of the output of a molecular regulatory network (for example, the activity of a transcription factor) on changing values of its input (for example, concentration of a growth factor in the extracellular medium).

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.

Unfolded protein response
The cellular response to the accumulation of misfolded proteins in the endoplasmic reticulum.

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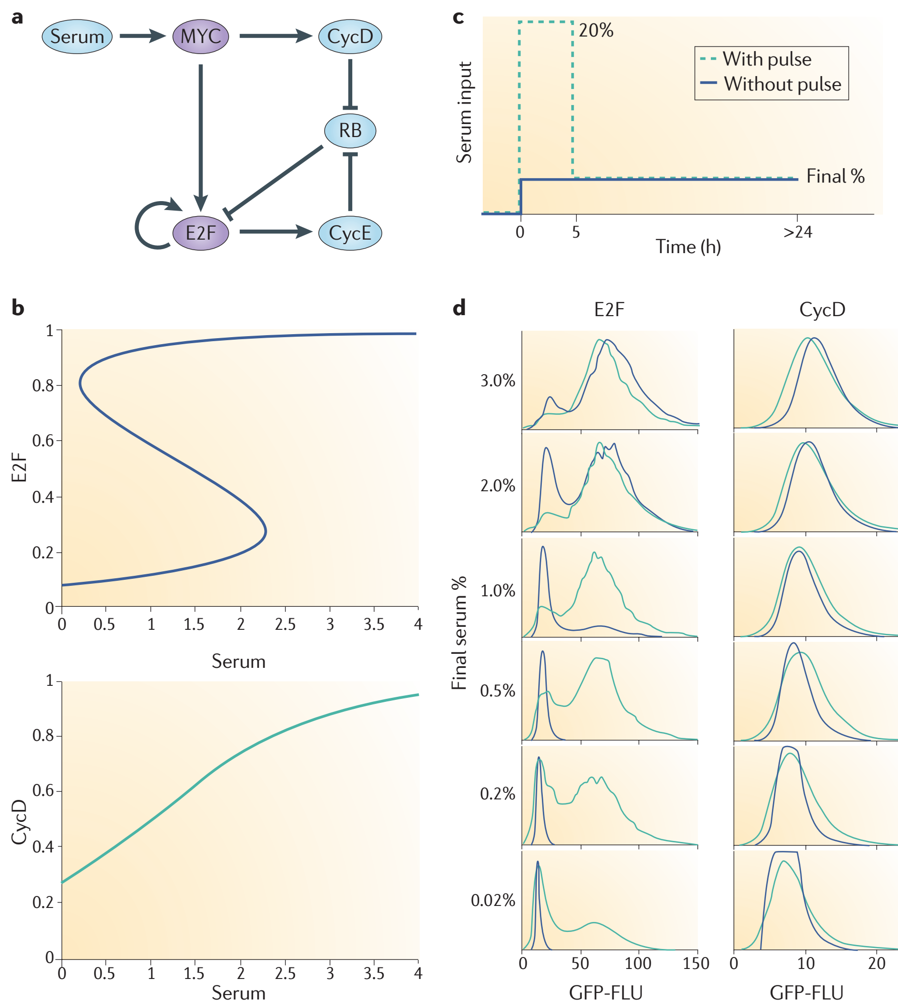

Figure 2 | **Bistable switch controlling the G1-to-S phase transition in mammalian cells.** **a** | 'Wiring' diagram for the bistable switch for G1-to-S phase transition. Cyclin E (CycE)–cyclin-dependent kinase complex promotes the transition of mammalian cells from the G1 phase of the cell cycle into the S phase. Quiescent cells are arrested in G1 by RB, which binds to and inhibits E2F, a family of transcription factors, some of which can promote cyclin E 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 (CycD)–cyclin-dependent kinase complex. After the G1–S phase transition is made, RB is maintained in its inactive (phosphorylated) form by cyclin E and by cyclin A- and B-dependent kinases that are active in the S, G and M phases (not shown). Quiescent cells, which have only small amounts of cyclin D, can be induced to proliferate by transcription factors (such as MYC, FOS and JUN) that are upregulated by growth factors in serum. These transcription factors promote the expression of both cyclin D and E2F genes, and E2F proteins upregulate their own transcription. **b** | Signal–response curves for E2F transcription factors and cyclin D. The wiring diagram in panel **a** is converted into a set of nonlinear differential equations (Supplementary information S1 (text)), and the steady-state levels of cyclin D and E2F are plotted as functions of serum concentration in the growth medium. Although cyclin D levels increase smoothly with serum concentration (and therefore act as a 'rheostat'), the E2F distribution exhibits a bimodal dependence on serum concentration (and therefore acts as a 'toggle switch')[41,43]. **c,d** | Experimental verification in rat embryonic fibroblasts (REF52 cells)[41]. Blue curves: serum concentration is raised from 0 to a final serum percentage. Green curves: serum concentration is raised to 20% for 5 hours, then lowered to a final serum percentage. The production of green fluorescent protein (GFP) is driven by an E2F gene promoter (left) or a cyclin D gene promoter (right). GFP fluorescence (FLU) measures the activities of these two promoters. Histograms of cyclin D gene expression shift smoothly up and down with the final serum percentage. E2F histograms show a bimodal dependence of gene expression on the final serum percentage between ~0.2% and 2%. There is a distinct hysteresis effect in the E2F response: on shifting serum levels up (blue curves), bistability is not observed until the serum level exceeds ~1%, but on shifting serum levels down (green curves) after cells have adapted to 20% serum, bistability is maintained to serum levels <0.2%. Parts **c** and **d** are reproduced, with permission, from REF. 41 © (2008) Macmillan Publishers Ltd. All rights reserved.

mitochondria-derived activator of caspase (SMAC; also known as DIABLO) to the cytoplasm, where cytochrome *c* promotes activation of 'executioner' caspases and SMAC neutralizes the inhibitor of apoptosis (IAP) proteins that inhibit caspases[55].

Based on our models (FIG. 3 and Supplementary information S2 (text)), the apoptosis switch is in the OFF or ON position depending on the balance between BCL-2-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).

The question of whether apoptosis is controlled by a bistable 'decision' module has generated considerable discussion, and the biochemical basis of such a module remains open to debate[51,53–56,58–62]. These contradictory viewpoints show 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-2–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 to still be functional in most cancers but more difficult to engage. For instance, in breast cancer cell lines, ER-mediated signalling upregulates anti-apoptotic proteins, including BCL-2 (REFS 23,63,64), BCL-W (also known as BCL-2-like protein 2)[64] and BCL-3 (REF. 65), making it harder to trigger apoptosis. Endocrine therapy, by inactivating the 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 upregulates genes encoding repair enzymes and p21 (also known as CIP1 and CDKN1A), which binds to and inhibits

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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, p53 upregulates production of BH3 proteins in an attempt to activate the apoptosis module. Whether apoptosis occurs or not depends on the levels of BH3 proteins relative to the levels of BCL-2 like proteins, thereby integrating the influences of apoptotic 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 FIGS 2,3, 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 survival mechanism, autophagy, which is thought to provide a steady supply of energy and raw materials by degrading the cell's own proteins and lipids[70]. Unremitting stress can lead to cell death, either by excessive autophagy or by activation of apoptosis[71].

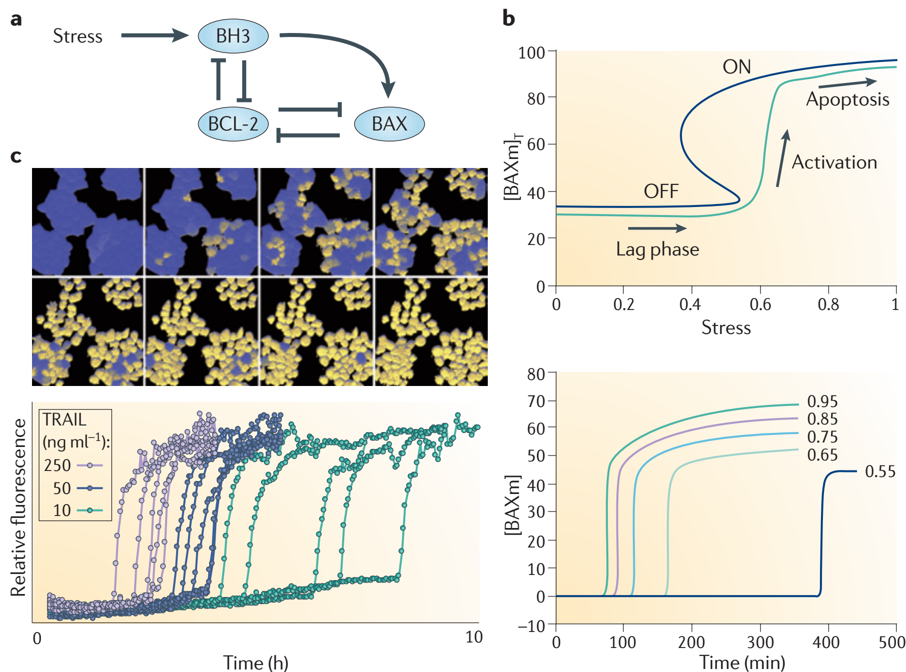

Figure 3 | **Bistable switch controlling apoptosis in mammalian cells. a** | 'Wiring' diagram for the bistable switch controlling apoptosis. Programmed cell death is triggered by activation of BAX proteins in the outer membrane of mitochondria. Active BAX causes the membrane to become permeable to proteins, such as cytochrome *c* and second mitochondria-derived activator of caspase (SMAC; also known as DIABLO), which induce the activation of proteases (caspases) and other hydrolytic enzymes that disassemble the macromolecules of the cell. BAX is activated by BH3-only family proteins and kept inactive by binding to BCL-2-family proteins. BH3 proteins also bind to BCL-2-family proteins[55]. **b** | Signal–response curve. A mathematical model of the wiring diagram is presented in Supplementary information S2 (text). Top: steady-state concentration (blue curve) of total membrane-bound BAX, $[BAXm]_T = [BAXm] + [BAXm–BCL\text{-}2]$, as a function of [Stress]. For intermediate levels of stress, the network has two stable steady states: an OFF state with a low total level of BAXm, all of it in complex with BCL-2 (that is, $[BAXm] \approx 0$); and an ON state with a high total level of BAXm, most of it not in complex with BCL-2 (that is, $[BAXm] \approx [BAXm]_T$). Bottom: time course of active BAX. Each simulation is started in the naive 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 green 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)[51]. Top: cells are treated with 50 ng ml−1 tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) and assayed for caspase activity by cleavage of an artificial substrate manufactured by the cell. Cells with high caspase activity are pseudo-coloured yellow. There is a 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. Part **c** is reproduced from REF. 51.

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 hydrolysed 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, BCL-2-interacting protein. When not bound to BCL-2, BECN1 participates in a multi-protein complex that initiates the earliest stages of autophagosome assembly[70–72].

In FIG. 4 we propose a simple model for the initiation of autophagy (for details, see Supplementary information S3 (text)). In this model, autophagy is regulated not as a toggle switch (as in FIGS 2,3) but as a rheostat; the levels of autophagy increase smoothly as stress increases. As autophagy increases, BCL-2 is released from its association with BECN1 and with the inositol 1,4,5-trisphosphate receptor (IP3R). The results can be variable and include survival (moderate autophagy and inhibition of apoptosis), 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 UPR module. The accumulation of unfolded proteins in the endoplasmic reticulum causes a characteristic response[73] 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 literature[73–76]. In FIG. 5 and Supplementary information S4 (text), we present a simplified model of the 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-oestrogens[19,77].

Signalling 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

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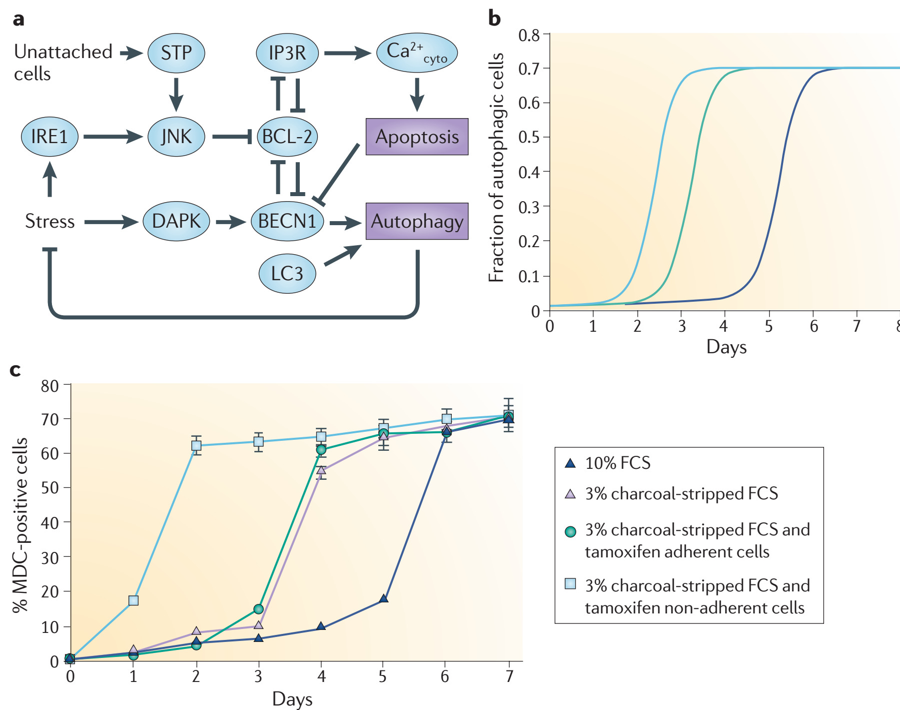

Figure 4 | **The interplay between autophagy and apoptosis. a** | 'Wiring' diagram for interplay between autophagy and apoptosis. In response to stress, both beclin 1 (BECN1) and BCL-2 are phosphorylated, causing the BCL-2–BECN1 complex to dissociate[103,104]. BECN1 is phosphorylated by death-associated protein kinase (DAPK), and BCL-2 is phosphorylated by JUN N-terminal kinase (JNK), a downstream target of the inositol-requiring protein 1 (IRE1; also known as ERN1) arm of the unfolded protein response (UPR)[75,103,104]. Detachment from the extracellular matrix provides an additional stress to the cells[105], which is transmitted to JNK by a signal transduction pathway (STP). Free BECN1 participates with other components, such as microtubule-associated protein 1 light chain 3α (LC3; also known as MAP1LC3A and ATG8), in initiating autophagy[71]. Autophagy can suppress the stress signal by providing the cell with ATP and raw materials for new protein synthesis. BCL-2 phosphorylation also allows the inositol 1,4,5-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 triggered[106]. Activated caspases cleave BECN1 and turn off autophagy[107]. Hence, under low stress conditions, autophagy promotes cell survival; at moderate stress, it may lead to autophagic cell death; and under conditions of high cellular stress, calcium release may stimulate apoptosis by the intrinsic (mitochondrial) pathway. **b** | Numerical simulations of interplay between autophagy and apoptosis. The wiring diagram is converted into a set of ordinary differential equations (Supplementary information S3 (text)), and the fraction of cells predicted to stain positive for autophagic vesicles is plotted as a function of time. The light blue curve shows detached cells under high stress, the green curve shows adherent cells under moderate stress and the blue curve shows detached cells with no stress. **c** | Experimental evidence in MCF-7 cells (breast cancer cell line)[105]. The percentage of cells that took up monodansylcadaverine (MDC) into autophagic vacuoles on days 1–7 was analysed. Non-adherent cells in 10% fetal calf serum (FCS) underwent autophagy by day 6. Non-adherent cells in the absence of oestrogen (3% charcoal-stripped FCS) undergo autophagy by day 4. Cells in 3% charcoal-stripped FCS with tamoxifen to inhibit oestrogen receptor function undergo autophagy by day 4. Non-adherent cells in 3% charcoal-stripped FCS with tamoxifen undergo autophagy by day 2. Ca^{2+}_{cyto}, Ca^{2+} in the cytoplasm. Part **c** is reproduced, with permission, from REF. 105 © (2007) Macmillan Publishers Ltd. All rights reserved.

of functional modules. Nonetheless, we must take into account that there is substantial crosstalk between modules, such as the apparent mutual inhibition between autophagy and apoptosis[71]. 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 signalling 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; these processes include the activation of a range of oestrogen-suppressed survival and proliferation genes, such as those involved in EGF signalling[81–86]. Interestingly, MCF-7 cells can be divided into two subgroups after the withdrawal of oestrogen[65]: most cells retain an absolute dependency on oestrogen and die as a result of the treatment, but some cells become oestrogen-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[87–89], then the acquired resistance can be reversed. For example, a population of MCF-7 cells that overexpress the EGF receptor (EGFR) or ERBB2 exhibit a bimodal distribution of receptors (FIG. 6c), and this distribution pattern can be reversibly controlled by manipulating the exposure of the cells to oestrogen[87,88]. We take these observations as evidence for a bistable survival switch that works through crosstalk between ER and EGF signalling pathways. Although little is known about how it works, mutual inhibition between these two pathways is likely to be a source of bistability. In FIG. 6 and Supplementary information S5 (text) we present a simple model that could account for the effects of oestrogen withdrawal on MCF-7 cells.

Crosstalk in cell signalling networks generates a large selection of discrete, stable and self-organized states; this creates a degree of cell-fate plasticity, which 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 utmost importance for designing more effective cancer treatment strategies.

Present realities and future directions

Mathematical modelling of intracellular molecular regulatory networks is an essential part of a systems approach to cancer biology[90]. Intuitive reasoning must be complemented by mathematical models when

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the molecular regulatory network under consideration is large, complex and interconnected and when we are dealing with quantitative aspects of signalling and control[91]. 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 that is amenable to computer simulation, mathematical analysis and detailed comparison to experimental data. By computing the behaviour of the model under various experimental conditions and comparing these simulations to the observed behaviour of cells, we can determine whether our hypothetical molecular mechanism is sufficient to account for the known behaviour of cells. If and when our model passes this first test ('post-diction'), we can use it to predict the behaviour of cells under novel experimental conditions, and use these quantitative predictions to test the efficacy of the model. Even when 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 to track down the origin of the problem (or problems) and consider alternative hypotheses.

Mathematical modelling of intracellular control systems related to breast cancer development, although still in its infancy, is beginning to provide some useful insights. For example, a sophisticated model of p53 signalling in MCF-7 cells successfully predicted a novel role for WIP1 (also known as protein phosphatase 1D) in a negative feedback loop from p53 to an upstream kinase in the DNA damage signalling pathway[92]. A recent model of the ERBB2–ER signalling network identified novel drug targets for trastuzumab-resistant cells[93]. A dynamic model of combinatorial cancer therapy suggested promising treatment strategies that were subsequently verified experimentally[94].

In this Opinion article, we have presented a roadmap for the mathematical modelling component of an integrative, systems biology of endocrine responsiveness in ER+ breast cancer. The hard work is yet to be done: researchers will need to formulate and verify models, estimate kinetic parameters, make non-obvious predictions and test 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 signalling, programmed cell death and the UPR suggest that there are no fundamental barriers to accurate, predictive models of complex control systems in mammalian cells. However, effective modelling is hampered by many substantial genetic and phenotypic differences among different types of mammalian cells. Extending models to cancer cells,

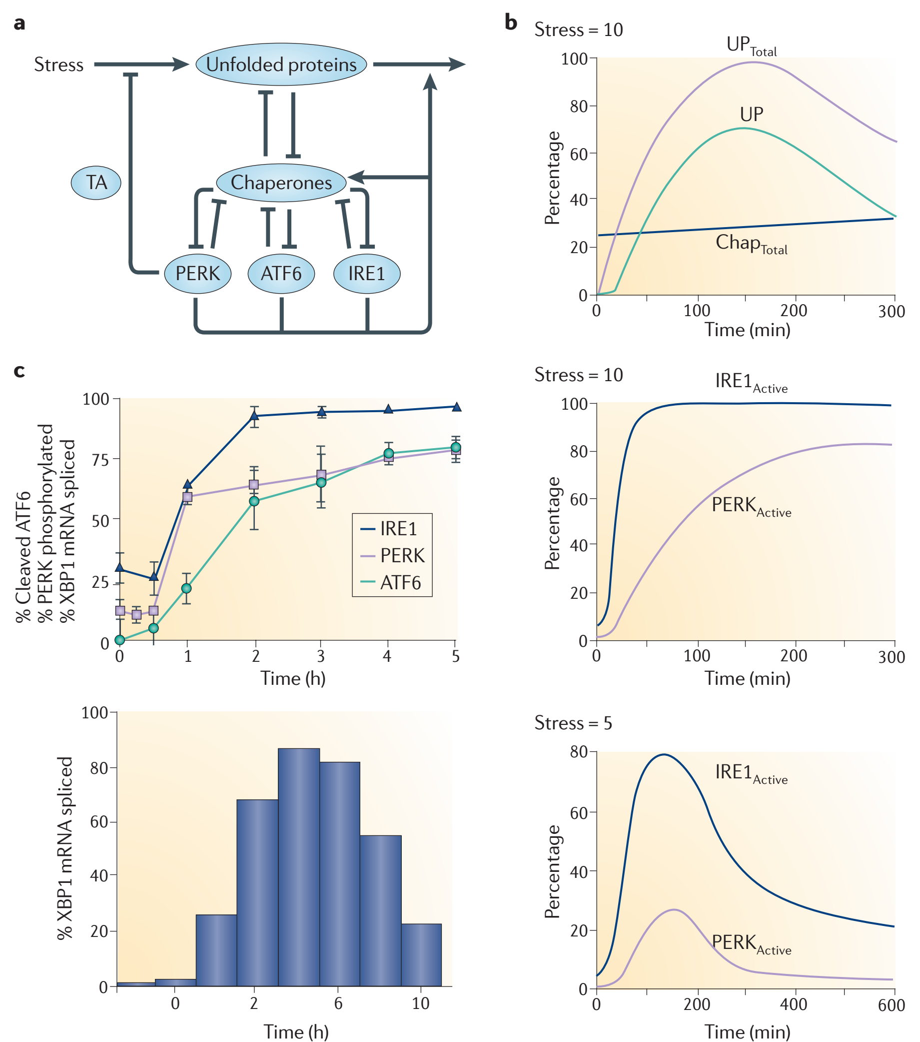

Figure 5 | **The unfolded protein response in mammalian cells. a** | 'Wiring' diagram for the unfolded protein response (UPR). The UPR is a coordinated cellular programme that is induced by the accumulation of unfolded and misfolded proteins in the lumen of the endoplasmic reticulum[73]. Increased levels of unfolded or misfolded proteins are brought down by chaperones, foldases, oxidoreductases, glycosylases and proteases (we refer to all of these components simply as chaperones). As unfolded proteins pull chaperones away from the lumenal domains of PRKR-like endoplasmic reticulum kinase (PERK; also known as EIF2AK3), activating transcription factor 6 (ATF6) and inositol-requiring protein 1 (IRE1; also known as ERN1), these three proteins upregulate the expression of certain genes that reduce the stress and increase the protein folding capacity of the endoplasmic reticulum. **b** | Numerical simulations computed from the mathematical model in Supplementary information S4 (text). At time 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 (UP), total chaperone, and protein species not bound to chaperones ($IRE1_{Active}$ and $PERK_{Active}$). The response to a stress of 5 arbitrary units applied at time 0 is also shown for $IRE1_{Active}$ and $PERK_{Active}$. **c** | Experimental verification in non-cancerous cells. Time courses of the three stress sensors after treatment of Chinese hamster ovary (CHO) cells with 10 µg ml^{-1} of tunicamycin to induce protein misfolding[108]. Time course of IRE1 activity (assayed as % splicing of X-box-binding protein 1 (XBP1) mRNA) in human embryonic kidney 293 cells treated with 5 µg ml^{-1} of tunicamycin[109]. TA, translation attenuation. The top graph in part **c** is reproduced, with permission, from REF. 108 © (2006) American Society for Cell Biology. The bottom graph in part **c** is reproduced from REF. 109.

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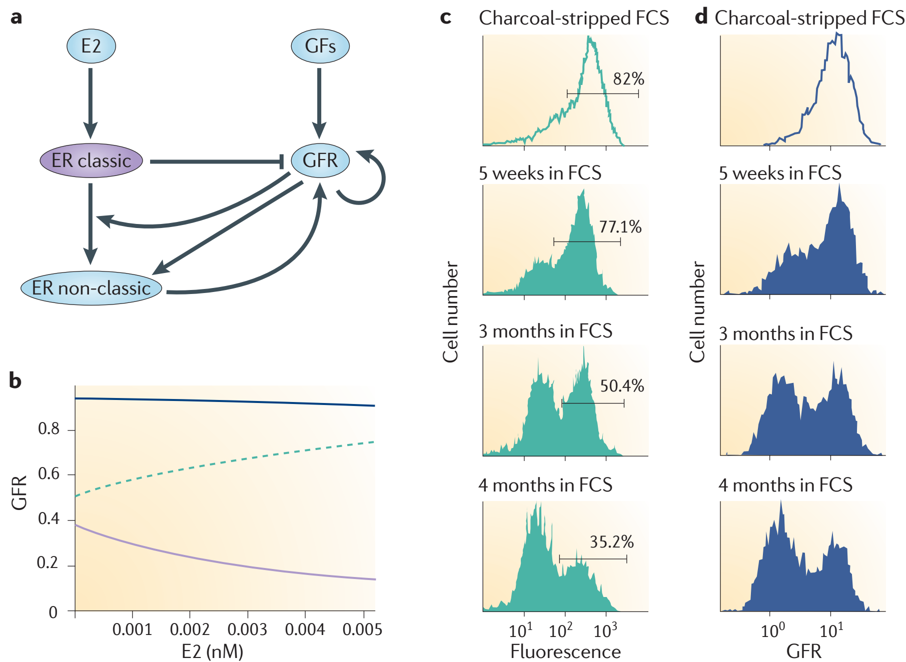

Figure 6 | **Crosstalk between oestrogen receptor and epidermal growth factor signalling pathways. a** | 'Wiring' diagram for crosstalk between oestrogen receptor (ER) and growth factor (GF) signalling. A growth factor receptor (GFR) and its downstream signalling network are suppressed by ligand-dependent ER signalling ('ER classic') but promoted by ligand-independent ER signalling ('ER non-classic'). Increased GF signalling after oestrogen (E2) withdrawal can shift ER from classical to non-classical signalling. GF signalling can upregulate its own activity. **b** | Signal–response curve for GFR as a function of E2. Differential equations describing the wiring diagram are provided in Supplementary information S5 (text). 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 dashed line indicates a branch of unstable steady states). **c** | Experimental evidence for crosstalk between ER and the epidermal growth factor (EGF) pathway[88]. The fluorescent activated cell sorting (FACS) plots in part **c** show a bimodal distribution of ERBB2 expression in a monoclonal culture of ERBB2-overexpressing MCF-7 cells induced by a change in [E2]. The plots show the number of cells expressing a certain abundance of ERBB2 as detected by fluorescent antibody. Cells are initially grown on charcoal-stripped fetal calf serum (FCS) for 5 weeks (or longer) to deplete them of E2, resulting in a single population of cells expressing high levels of ERBB2 (top plot). Replacing charcoal-stripped FCS with FCS (FCS contains E2) leads to the emergence over time (shown at 5 weeks, 3 months and 4 months) of a second population of cells expressing a low level of ERBB2 (indicated by the peak on the left). Similar results have also been observed in EGF-overexpressing MCF-7 cells[87]. **d** | Model simulation of crosstalk between ER and ERBB2. In Supplementary information S5 (text), noise terms are added to the differential equations to take stochastic effects into account. Two thousand cells were grown in the absence of E2 for 3.3 weeks, which resulted in a single population of cells expressing high levels of ERBB2 (top plot). Adding in FCS (corresponding to 5 pM of E2) leads to the emergence over time (shown at 5 weeks, 3 months and 4 months) of a second population of cells expressing a low level of ERBB2 (indicated by the peak on the left). The pattern of ERBB2 expression is similar to the results shown in panel **c**. Part **c** is reproduced, with permission, from REF. 88 © (1995) Springer.

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 that control 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 modelling intracellular control systems stems from the fact that the behaviour of populations of cells (for example, their graded response to drug treatment) may not reflect the behaviour of single cells (in this example, an all-or-none decision in response to the drug). At present, modellers are still struggling with how best to cope with all of these competing issues.

In addition, there are other relevant theoretical considerations that we have not described in this article. First, realistic models of molecular regulation must take into account the compartmentalization of eukaryotic cells. Second, 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'). Third, our models only bridge the scales from molecular networks to cell physiology. Breast tumours exist in a complex microenvironment that affects the dynamic signalling within and among cancer cells. Modelling these effects adds new layers of complexity. Other kinds of mathematical models are needed to describe how tumour cells are organized into multicellular tissues that interact with the extracellular matrix, recruit vasculature and eventually metastasize 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 (for example, the cell cycle) and of intercellular communication (for example, WNT signalling)[101,102].

We expect that these modelling challenges can be overcome and that a new generation of mathematical models will provide new insights into the molecular foundations of endocrine responsiveness in breast cancer.

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Competing interests statement

The authors declare no competing financial interests.

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