Supplementary document to:

Modeling the estrogen receptor to growth factor receptor signaling switch in human breast cancer cells

Chun Chen[||], William T. Baumann[‡], Robert Clarke[§], John J. Tyson*

*Department of Biological Sciences, [‡]Department of Electrical & Computer Engineering, and [||]Graduate Program in Genetics, Bioinformatics and Computational Biology, Virginia Polytechnic Institute & State University, Blacksburg VA 24061, USA

[§]Lombardi Comprehensive Cancer Center, Georgetown University School of Medicine, Washington DC 20057, USA

* Corresponding author. Fax: +1 540 231 9307.
E-mail address: tyson@vt.edu.

Schematic illustrations of the experimental observations

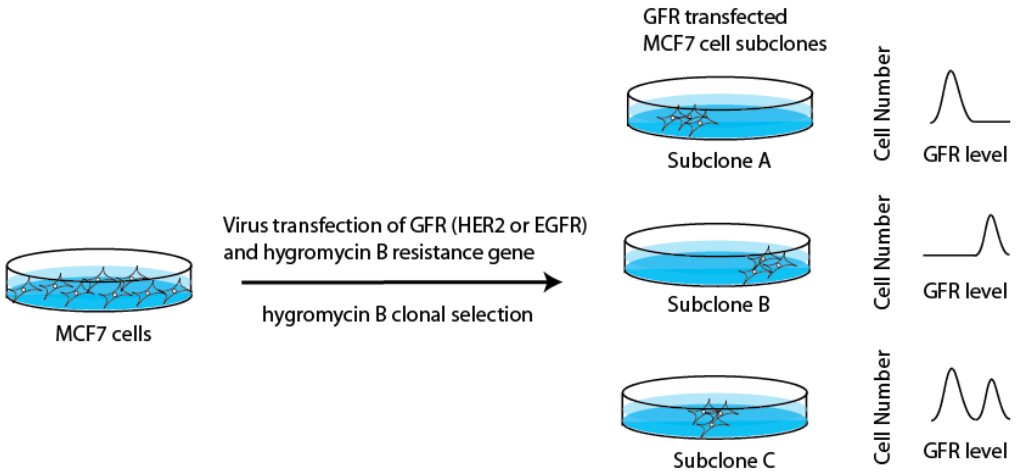

Figure S1. Three different distribution patterns of GFR in sub-clones of MCF7 cells transfected with GFR (HER2 or EGFR) [1, 2].

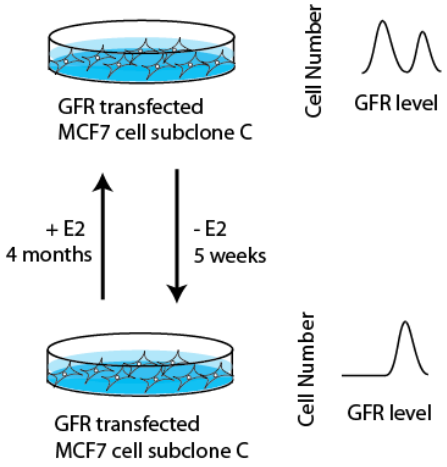

Figure S2. E2 reversibly modifies the bimodal distribution of GFR in a GFR-transfected MCF7 subclone [1, 2]. E2 withdrawal switches on GFR expression within weeks, whereas E2 addition takes months to switch off GFR expression [1, 2].

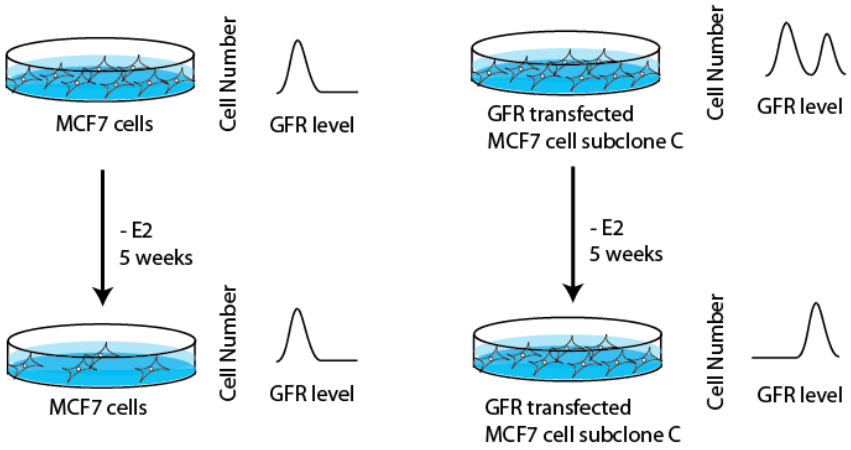

Figure S3. E2 withdrawal can up-regulate GFR expression within 5 weeks in GFR-transfected MCF7 cells, but fails to do so in wild type MCF7 cells [1, 2].

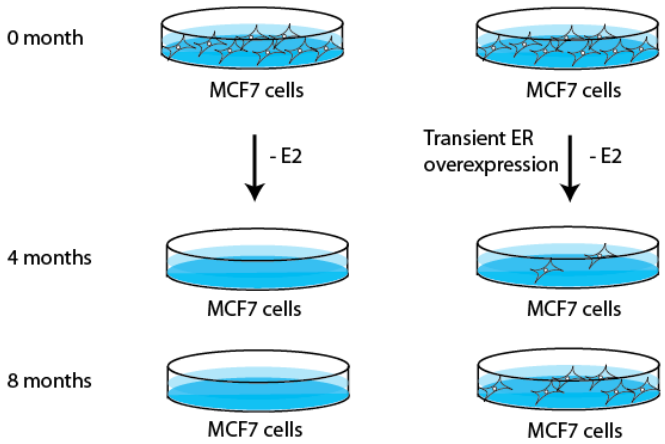

Figure S4. Transient ER overexpression in MCF7 cells can switch on the GFR pathway and promote E2-independent growth [3].

Materials and Methods

Model Implementation

We postulate a highly condensed model of the interaction between ER and GFR (Fig. 1A in manuscript). The protein level of GFR is down-regulated by E2:ER complex. After E2 withdrawal, GFR expression is released from inhibition, and its downstream kinases activate E2-independent ER-P. ER-P can activate and stabilize the GFR pathway, creating a positive feedback loop. In addition, GFR further activates transcription factors such as NFκB, promoting a series of epigenetic changes contributing to increased GFR expression and establishing another positive feedback loop. For simplicity, we combine the epigenetic factors contributing to GFR expression into the quantity 'EPI'. 'E2ER' and 'ERP' are used to represent E2:ER and ER-P.

The wiring diagram in Fig. 1A was translated into ordinary differential equations (ODEs) to enable simulation and analysis. In these equations, the levels of GFR, EPI, E2ER and ERP are represented by italicized variables: *GFR*, *EPI*, *E2ER* and *ERP*. The rates of change for *E2ER* and *ERP* are considered fast compared to the rates for *GFR* and *EPI*, so for simplicity only *GFR* and *EPI* are described by differential equations. E2ER and ERP are assumed to be proportional to *ERT,* a parameter we use to vary the total level of ER in the cell. Note that E2-binding and phosphorylation of ER are not necessarily mutually exclusive. Moreover, we assume that *GFR* and *EPI* have a dynamical range of about 10-fold, so on a log10 scale they vary between 0 and 1. Since our model is phenomenological in nature, we do not use standard reaction rate equations. Rather, we apply a formalism that allows us to capture complex dependencies in a simple manner [4]. The model equations are:

$$\frac{dEPI}{dt} = \gamma_{\mathrm{EPI}} \cdot \left(H\left(W_{\mathrm{EPI}}\right) - EPI \right) \tag{S1}$$

$$\frac{dGFR}{dt} = \gamma_{\mathrm{GFR}} \cdot \left(H\left(W_{\mathrm{GFR}}\right) - GFR \right) \tag{S2}$$

$$E2ER = H\left(W_{\mathrm{E2ER}}\right) \cdot ERT \tag{S3}$$

$$ERP = H\left(W_{\mathrm{ERP}}\right) \cdot ERT \tag{S4}$$

where

$$H(W) = \frac{1}{1+e^{-W}} \tag{S5}$$

$$W_{\mathrm{EPI}} = \omega_{\mathrm{EPI}} + \omega_{\mathrm{EPI,GFR}} \cdot GFR \tag{S6}$$

$$W_{\mathrm{GFR}} = \omega_{\mathrm{GFR}} + \omega_{\mathrm{GFR,EPI}} \cdot EPI + \omega_{\mathrm{GFR,E2ER}} \cdot E2ER + \omega_{\mathrm{GFR,ERP}} \cdot ERP + \omega_{\mathrm{GFR,GFRover}} \cdot GFRover \tag{S7}$$

$$W_{\mathrm{E2ER}} = \omega_{\mathrm{E2ER}} + \omega_{\mathrm{E2ER,E2}} \cdot E2 \tag{S8}$$

$$W_{\mathrm{ERP}} = \omega_{\mathrm{ERP}} + \omega_{\mathrm{ERP,GFR}} \cdot GFR \tag{S9}$$

The parameters γ_{EPI} and γ_{GFR} determine the rate at which *EPI* and *GFR* approach their steady state values. $H(W)$ is a sigmoidal function. W_i is the net activation or inhibition of species i (i = EPI, GFR, E2ER and ERP), and ω_i determines whether species i is 'on' or 'off' when there is no other factor regulating i. $\omega_{i,j}$ indicates the influence of species or stimulus j (j = EPI, GFR, E2ER, ERP, GFRover, E2) on species i. A description of the variables used in our model is given in Table S1.

The parameter values, listed in Table S2, were obtained by manually choosing values to fit the experimental observations. Initially, the parameters for the deterministic part of the model where chosen to obtain a bifurcation diagram as a function of *GFRover* that exhibited three distinct patterns of expression and simultaneously a bifurcation diagram as a function of *E2* that allowed GFRs to switch on when E2 was withdrawn. Then the noise parameters where chosen to capture the experimentally observed timing of the on and off transitions of GFR. The noise and deterministic parameters are not independent and so ultimately all the parameters were adjusted in concert to match the experiments. Since we are modeling this system at a high level of abstraction, none of our parameters are directly related to measurable physical rate constants of the system, but rather are phenomenological parameters that match the phenotypic performance of the system. We used the program XPP-AUT, available freely at http://www.math.pitt.edu/~bard/xpp/xpp.html, to simulate the model and draw bifurcation diagrams.

Table S1. Model variables and their descriptions.

Variables	Range	Description
EPI	[0,1]	Epigenetic factors contributing to GFR expression
GFR	[0,1]	GFR expression level
E2ER	[0, *ERT*]	E2-dependent E2:ER activity
ERP	[0, *ERT*]	E2-independent ER-P activity

Table S2. Model parameters, descriptions, and numerical values.

Parameters	Description	Value
γ_{EPI}	Rate of EPI reaching its steady state	3×10^{-4} min^{-1}
γ_{GFR}	Rate of GFR reaching its steady state	5×10^{-2} min^{-1}
ω_{EPI}	Basal inhibition of EPI	−1.92
ω_{GFR}	Basal inhibition of GFR	−4
ω_{E2ER}	Basal inhibition of E2ER	−2.1
ω_{ERP}	Basal inhibition of ERP	−1.5
$\omega_{EPI,GFR}$	EPI activation by GFR	6
$\omega_{GFR,EPI}$	GFR activation by EPI	5
$\omega_{GFR,E2ER}$	GFR inhibition by E2ER	−2
$\omega_{GFR,ERP}$	GFR activation by ERP	1.85
$\omega_{GFR,GFRover}$	GFR activation by GFRover	0.15
$\omega_{E2ER,E2}$	E2ER activation by E2	3
$\omega_{ERP,GFR}$	GFR activation by ERP	3
E2	E2 level	1 (normal); 0 (E2-depleted)
ERT	Total ER level	1 (normal); >1 (ER-overexpressed)
GFRover	Excess GFR in transfected cells	0 (normal); >0 (GFR-transfected)

Stochastic simulation

To account for stochastic effects in the model, noise terms are added to the differential equations for *EPI* and *GFR*, while the algebraic equations are left unchanged, for simplicity. The Langevin equation for variables *i* (*i* = *EPI* or *GFR*) takes the form:

$$\frac{di}{dt} = \gamma_i \cdot (s_i - i) + F_i(t) \tag{S11}$$

where s_i defines the steady state level of i and $F_i(t)$ is a Gaussian white noise process. The equilibrium second moment of the variable i, $\langle (s_i - i)^2 \rangle_{eq} = \theta_i$, is related to γ_i and the second moment of the noise by a fluctuation-dissipation theorem [5, 6]:

$$\langle F_i(t) F_i(t') \rangle_{eq} = 2 \cdot \gamma_i \cdot \theta_i \cdot \delta(t - t') \tag{S12}$$

We choose a suitable value for θ_i and rewrite Eq. 14 as:

$$\frac{di}{dt} = \gamma_i \cdot (s_i - i) + \sqrt{2 \cdot \gamma_i \cdot \theta_i} \cdot \zeta_i(t) \tag{S13}$$

where $\zeta_i(t)$ is a temporally uncorrelated, statistically independent, Gaussian white noise process formally defined by $\zeta_i(t) \equiv \lim_{dt \to 0} N(0, 1/dt)$ with $\langle \zeta_i(t) \zeta_i(t') \rangle = \delta(t - t')$.

The Langevin equations are integrated and propagated by the explicit method:

$$i(t + \Delta t) = i(t) + \gamma_i \cdot (s_i - i) \cdot \Delta t + \sqrt{2 \cdot \gamma_i \cdot \theta_i \cdot \Delta t} \cdot \eta_i(t) \tag{S14}$$

where the $\eta_i(t)$ are independent normal random variables. We used $\theta_{EPI} = 0.0008$ and $\theta_{GFR} = 0.01$ to fit the experimental data. The stochastic simulations were performed in Matlab Version 7.9.0.

References

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6. Zwanzig, R., *Dynamic Disorder - Passage through a Fluctuating Bottleneck.* Journal of Chemical Physics, 1992. **97**(5): p. 3587-3589.