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The Core Control System of Intracellular Iron Homeostasis: A Mathematical Model

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Abstract

Iron is a metal essential for cellular metabolism. However, excess iron available for reactions contributes to the formation of dangerous reactive oxygen species, such as the hydroxyl radical, via the Fenton reaction. Therefore, intracellular iron levels are tightly constrained by a control system of proteins. This paper contains a mathematical model, in the form of a system of 5 ordinary differential equations, of the core of this control system, including the labile iron pool as well as proteins that regulate uptake, storage, and export and are connected through negative feedback loops. The model is validated using data from an overexpression experiment with

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Author Contributions

JC led the development of the model and its mathematical analysis. AK, PN, IW, and BL contributed to model development and the steady state analysis. ZD designed and executed ferroportin overexpression experiment. PM contributed to model analysis. VH contributed to the identification of the core network to be modeled. FT and SA contributed biological expertise to the project. ST provided biological expertise and data from her laboratory, used in the paper. RL developed the concept of the project, provided partial funding, and directed the project. All authors contributed to the final version of the manuscript.

cultured human breast epithelial cells. The parameters in the mathematical model are not known for this particular cell culture system, so the analysis of the model was done for a generic choice of parameters. Through a mixture of analytical arguments and extensive simulations it is shown that for any choice of parameters the model reaches a unique stable steady state, thereby ruling out oscillatory behavior. It is shown furthermore that the model parameters are identifiable through suitable experiments.

Keywords

Iron metabolism; Breast epithelial cells; Differential equations

1. Introduction

Iron is an essential component for any aerobic organism and is required for oxygen transport, cellular respiration, and many other metabolic processes. But iron is also a redox active element that can facilitate the formation of dangerous molecules, such as the hydroxyl radical, a highly reactive radical that can damage DNA, lipids, and proteins. Dysregulation of iron homeostasis has been implicated in a wide variety of diseases, ranging from cancer to inflammatory and neurodegenerative diseases (see, e.g., [1]). Thus, iron metabolism and storage are highly regulated at both the organismal and the intracellular levels. A plethora of new insights in recent years has revealed a complex control network of proteins and other molecules involved in iron homeostasis at the organismal as well as the cellular level. In the review article [2] authors have assembled an intracellular network consisting of 151 chemical species and 107 reactions and transport steps, including cell type specific subnetworks. The article also identifies a core regulatory network that contains several intertwined feedback loops and includes the key regulatory mechanisms of iron homeostasis.

In the present paper we focus on this core control system in the form found in breast epithelial cells. This choice of cell type is motivated by our interest in the role of intracellular iron homeostasis in the pathogenesis of breast cancer. It has been shown that some of the proteins involved are differentially regulated in breast cancer cells and can be used as prognostic markers [3]. Indeed, a recent editorial in *Lancet* argues that differences in iron metabolism may be key to both development and recurrence of breast cancer [4]. It has been known for years that individuals who accumulate excess iron have an increased cancer incidence [5, 6, 7, 8]. In addition, high levels of dietary iron accelerate the growth of breast tumors in animal models [9, 10]. An increase in transferrin receptor 1, a protein responsible for iron import, has been observed in breast and other cancers as early as the 1980s [11]. In fact, the transferrin receptor has been used as a targeting ligand in the design of anti-cancer drugs [12]. In a cell culture system, repression of ferritin, an iron storage protein, and activation of IRP2, an iron regulatory protein, was required for the transforming and proliferative effects of the c-myc oncogene [13]. Moreover, levels of the plasma membrane protein ferroportin, an iron export pump, are decreased in malignant breast cells [3]. The study reported in [3] also revealed that overexpressing ferroportin in breast cancer cells led to tumor suppression.

However, mechanisms underlying changes in iron metabolism during malignant progression, how they contribute to malignant processes, and whether they can be successfully exploited to therapeutic advantage remain unknown. Our ultimate goal is to construct predictive mathematical models of iron metabolism in both normal and malignant breast cells that will enable us to understand basic forces that drive changes in iron metabolism during malignant progression. In this article we present a mathematical model for the core control system in normal breast epithelial cells.

The key molecular species to be controlled is the labile iron pool, defined as the collection of weakly bound iron molecules in the cell. We also include the proteins responsible for iron import, export, and sequestration, together with the iron regulatory proteins, which regulate these three mechanisms in response to changing levels of the labile iron pool. We furthermore include the small molecule hepcidin, recognized as a key regulator of iron efflux. These molecules are connected through several intertwined feedback loops that control network dynamics. While this network represents only a small part of the network reconstructed in [2], an understanding of its dynamic properties is crucial for insights into the effect of perturbations of the larger network. We have used the model of intracellular iron homeostasis constructed in [14] as a guide. The authors focused primarily on the role of the iron sequestering protein ferritin and explored the possible dynamic regimes in different parts of the parameter space. Since part of the feedback structure of our network is missing in [14], their model differs substantially from ours.

The paper is organized as follows. Section 2 is an overview of intracellular iron homeostasis. In Section 3 we construct a mathematical model in the form of a system of ordinary differential equations and analyze its dynamics. In any given cell type most or all of the model parameters are unknown. Even if data from extensive time course experiments were available, there are well-known problems inherent in obtaining a quantitative model through parameter fitting (see, e.g., [15]). We have therefore opted instead for a detailed mathematical analysis of the model that extracts properties that are independent of parameter choices. In particular, in Section 3.3 we show analytically that the model has a unique steady state for any choice of parameter values, and we show through extensive numerical simulations that this steady state is stable. This agrees with experimental evidence that in the absence of perturbations cellular iron levels are tightly controlled [16]. Finally, in Section 4 we validate the model using data from experiments with mammary epithelial cells in culture. The paper concludes with a discussion in Section 5.

2. Control of Intracellular Iron Homeostasis

The key task of intracellular iron homeostasis is to tightly control the level of the so-called *labile iron pool*, abbreviated as LIP, the collection of weakly bound iron in the cell, which is available for a variety of interactions with other molecules. Cells have available mechanisms to import iron from the circulation to increase LIP, sequester iron using a storage protein, or exporting it through an iron efflux pump in order to decrease LIP. We briefly describe these here. Details can be found in [2].

The primary conduit for iron import in breast epithelial cells is the membrane protein transferrin receptor 1, TfR1. Iron is found in the circulation mostly bound to transferrin, Tfn, a transporter protein that has two binding sites for ferric iron, forming holotransferrin or diferric Tfn (Tfn bound to two iron atoms). Transferrin receptor 1 is a ubiquitous protein expressed on the cell surface, and binds to both. The resulting complex is internalized into acidified endosomes. Iron dissociates from Tfn in these endosomes and is then transported into the cytosol as ferrous iron. Tfn is then recycled to the cell surface. As mentioned earlier, excess free ferrous iron can be toxic to the cell. In the presence of hydrogen peroxide, ferrous iron can contribute to the formation of the dangerous hydroxyl radical through the Fenton reaction. The ferritin protein Ft oxidizes and sequesters excess ferrous iron into a ferrihydrite mineral core. Each Ft molecule can bind as many as 4500 iron atoms. The other primary mechanism to lower the level of the labile iron pool is iron efflux using the protein ferroportin, Fpn. Ferroportin is currently the only known mammalian iron exporter located on the plasma membrane and is expressed in a wide variety of human tissue types.

These different mechanisms are regulated by the iron regulatory proteins (IRPs) in response to changing LIP levels, using a very elegant mechanism, which acts post-transcriptionally and utilizes so-called iron responsive elements (IREs). IREs are nucleotide hairpins in the untranslated regions of mRNA of certain proteins involved in iron metabolism. There are two IRPs, IRP1 and IRP2, which are regulated differently by iron levels. The regulation by IRPs works as follows. IRP1 and IRP2 can bind to an IRE with high affinity when iron levels are low. If the IRE is located in the 5' UTR, then binding by IRP blocks translation. If the IRE is in the 3' UTR, on the other hand, the effect is a stabilization of the mRNA so more protein gets translated. When iron levels are high, both proteins lose their affinity for the IREs and their regulatory effect ceases and translation reverts back to levels of constitutive expression. The mRNAs for ferritin and ferroportin contain IREs in the 5' UTR. Thus, under low iron conditions their translation is reduced, resulting in the increased availability of intracellular iron. On the other hand, the iron importer TfR1 contains IREs in the 3' UTR, so that its translation is enhanced under low iron conditions. (For simplicity we will represent IRP1 and IRP2 as a single node, denoted by IRP.)

We also include hepcidin, Hpc, in the model, a hormone secreted in several cell types, including breast epithelial cells. Hepcidin binds to the iron exporter ferroportin, inducing its degradation and thus the prevention of iron export into the extracellular environment. The regulatory mechanism is based on the recruitment and activation of Jak2 following the binding of ferroportin to hepcidin; activated Jak2 phosphorylates ferroportin and targets it for degradation.

The resulting control system is pictured in Figure 1. We observe that there are three negative feedback loops present, each using the inhibitory edge from LIP to IRP. The three feedback loops involve TfR1, Ft, and Fpn, respectively. These feedback loops control the dynamics of the network, which can only be understood properly through a dynamic mathematical model. This is what we present in the next section.

3. Mathematical Model

As described, the core of the iron network consists of the labile iron pool (LIP), the iron import protein transferrin receptor 1 (TfR1), iron export/storage proteins ferroportin(Fpn)/ferritin(Ft) and iron regulatory proteins IRPs. We focus on these species as candidate key control points for the following reasons:

1. they participate in regulatory feedback loops;
2. regulatory interactions involving these species have been consistently observed in the literature;
3. they have been observed in multiple cell types, suggesting conservation of function;
4. they are experimentally tractable;
5. they include several species that have been directly shown to change in malignant cells, including TfR1, ferritin and ferroportin [3, 13, 11].

Although many species have been omitted in this core model, we believe it to be a reasonable starting point. In fact, it is essential to first understand this core system before being able to properly assess the effect of other components in the larger iron homeostasis network.

3.1. Model Assumptions

We list here the biological assumptions underlying the model.

- Once in the endosome, it is believed that ferric iron is reduced to ferrous iron by the transmembrane family of proteins (STEAP) and then transported into the cytoplasm by divalent metal transporter 1 (DMT1) [17]. However, the role of these proteins in iron metabolism has been defined in cell types that regulate systemic iron homeostasis, such as liver cells, macrophages, enterocytes, erythroid cells, and cells of the bone marrow [18]. Their role in peripheral tissues is less well studied. We restricted our initial model to proteins known to be expressed in breast cells: transferrin receptor 1 (TfR1), ferritin (Ft), ferroportin (Fpn), iron regulatory proteins (IRP's), and hepcidin (Hepc). Many more interactions are possible and indeed probable. We also note that we have not considered the potential impact of iron shuttling among organelles, largely because very little is known about the contribution of intracellular iron trafficking by proteins such as STEAP and DMT1 to iron management in breast epithelial cells. We anticipate that these and other considerations will require further refinements of our model as it evolves to more closely approximate the living cell.
- In our model ferritin is always bound to iron and is assumed to undergo natural degradation. As ferritin degrades, it releases iron that has been sequestered back into the labile iron pool. Indeed, several studies suggest that degradation of cytosolic ferritin by lysosomes is one of the mechanism by which iron is released from ferritin. Iron is then transported back into the cytosol [19, 20, 21]. Thus, our model assumes two processes that increase the labile iron pool: iron import by TfR1 and dissociation of iron from ferritin.
- We make the assumption that the amount of iron imported/exported is proportional to the amount of TfR1/Fpn present [22, 23].
- We represent both IRP's as active and as one single node in the network. Even though there is some evidence that IRP2 dominates regulation of iron homeostasis [24, 25], there is no evidence that IRP2 regulates a set of proteins different from that regulated by IRP1. Both IRP's are equally likely to regulate ferritin, ferroportin and transferrin receptor [26].
- We introduce two external control parameters, Fe_{ex} and Hep , into the model. Fe_{ex} represents the iron saturation level of extracellular transferrin and was first introduced in [14]. This parameter will be used in our simulations to study the effect of different saturation levels of diferric transferrin. Hepcidin binds to ferroportin, inducing its degradation, thus Hep is a constant that will be used to investigate its influence on inhibiting ferroportin. These parameters do not have a functional relationship in the model, that is Fe_{ex} and Hep do not change over time. Our rationale for this decision is that both parameters are external to the system, and since our model is not a systemic model, it is not necessary at this time to represent Fe_{ex} and Hep through differential equations.

3.2. The Model—The model consists of five non-linear ordinary differential equations describing changes in concentrations with respect to time for a specific component of the model. For convenience we represent the concentration of each protein and the level of the labile iron pool by: $x_1 = [LIP]$, $x_2 = [TfR1]$, $x_3 = [Fpn]$, $x_4 = [Ft]$, and $x_5 = [Active\ IRP's]$, that is, $x_1, \dots, x_5$ are state variables.

$$\begin{aligned}
 \dot{x}_1 &= \alpha_1 Fe_{ex} x_2 + \gamma_4 x_4 - \alpha_6 x_1 x_3 - \alpha_4 x_1 \frac{k_{54}}{k_{54} + x_5} \\
 \dot{x}_2 &= \alpha_2 \frac{x_5}{k_{52} + x_5} - \gamma_2 x_2 \\
 \dot{x}_3 &= \alpha_3 \frac{k_{53}}{k_{53} + x_5} - (\gamma_3 + \gamma_h Hep) x_3 \\
 \dot{x}_4 &= \alpha_4 x_1 \frac{k_{54}}{k_{54} + x_5} - \gamma_4 x_4 \\
 \dot{x}_5 &= \alpha_5 \frac{k_{15}}{k_{15} + x_1} - \gamma_5 x_5
 \end{aligned} \tag{1}$$

The model includes four key regulatory mechanisms: (a) The IRP's respond directly to the level of LIP; (b) translation of Ft and Fpn is negatively regulated by the IRP's; and (c) mRNA stability of TfR1 (and consequently levels of TfR1 protein) is positively regulated by the IRP's. We assume hyperbolic rates for these four mechanisms, which is appropriate here and is widely used for metabolic networks, where x_i is an activating/inhibiting state variable and k_{ij} is the activation threshold:

$$\frac{\alpha_j x_i}{k_{ij} + x_i} \text{ and } \frac{\alpha_j k_{ij}}{k_{ij} + x_i}, \tag{2}$$

for $i \in \{1, 5\}$, $j \in \{2, 3, 4, 5\}$ and α_j is the maximum production rate of the regulated protein. Each protein undergoes self-degradation, thus the linear term $\gamma_i x_i$, for $i = 2, \dots, 5$ represents the decay of x_i , where γ_i is a constant decay rate. Recall that *Hep* does not have a functional role in the model and may be viewed as a control parameter, hence the degradation of ferroportin induced by hepcidin is represented by the linear term $(\gamma_h Hep) x_3$. Here, γ_h is the maximum rate and *Hep* is fixed to be between 0 and 1. Iron import is represented by the term $\alpha_1 Fe_{ex} x_2$ with the rate α_1 proportional to x_2 , and iron export is represented by $\alpha_6 x_1 x_3$ with the rate α_6 .

Lastly, in Section 3.1 we have assumed that ferritin is always bound to iron, undergoes natural degradation and, as it degrades, releases iron that has been sequestered back into the labile iron pool. Thus, we may represent this mechanism as follows:

where $\beta = \frac{\alpha_4 k_{54}}{k_{54} + x_5}$ is described by Equation (2), and γ_4 is a constant decay rate.

We note that Figure 1 is a simplified representation of the core network and does not include some of the relations (e.g. self-degradation) and model assumptions used in the ODE model (1).

3.3. Qualitative Analysis—An equilibrium point of a dynamical system represents a stationary state for the dynamics. Solving System (1) for an equilibrium leads to a single cubic polynomial in terms of $x_1 = [\text{LIP}]$:

$$p(x_1) = ax_1^3 + bx_1^2 + cx_1 + d, \tag{3}$$

where

$$\begin{aligned}
 a &= \alpha_3 \alpha_6 \gamma_2 (\gamma_5^2) k_{52} k_{53} \\
 b &= \alpha_3 \alpha_6 \gamma_2 \gamma_5 k_{15} k_{53} (\alpha_5 + 2\gamma_5 k_{52}) \\
 c &= \gamma_5 k_{15} (-\alpha_1 \alpha_2 \alpha_5 Fe_{ex} (\gamma_3 + \gamma_h Hep) + \alpha_3 \alpha_6 \gamma_2 k_{15} (\alpha_5 + \gamma_5 k_{52})) k_{53} \\
 d &= -\alpha_1 \alpha_2 \alpha_5 Fe_{ex} (\gamma_3 + \gamma_h Hep) (k_{15})^2 (\alpha_5 + \gamma_5 k_{53}).
 \end{aligned}$$

Observation 1. *In our model all parameters are strictly positive real numbers. This implies that the coefficients of $p(x_1)$ are as follows: $a > 0$, $b > 0$ and $d < 0$, while c could be of either sign depending on the choice of parameters.*

Corollary 2. *For any choice of parameters, $p(x_1)$ has exactly one positive solution, while the other two solutions are either negative real or complex.*

Proof. First, suppose that we have one real solution and two complex solutions. Let r be the real solution and z be a complex solution. Then the equation can be factored as:

$$(x_1 - r)(x_1 - z)(x_1 - \bar{z}) = 0$$

The free term in this equation is given by $-r \cdot z \cdot \bar{z}$, where $z \cdot \bar{z} = |z|^2 \geq 0$. By Observation 1 the free term is negative, thus $r > 0$.

Now suppose that we have three real solutions. Let r_1 , r_2 and r_3 be three real solutions, then

$$(x_1 - r_1)(x_1 - r_2)(x_1 - r_3) = 0$$

The free term is given by $-r_1 \cdot r_2 \cdot r_3$ and it must be negative by Observation 1. This leads to exactly two conditions:

- i. all of the three roots are positive, or
- ii. one of the roots is positive and the other two roots are negative.

The quadratic term in the above equation is given by $-(r_1 + r_2 + r_3)$ and must be positive by Observation 1. This implies that $(r_1 + r_2 + r_3)$ is negative, which means Condition (i) is not possible. Therefore, Condition (ii) must hold, i.e. one of the roots is positive and the other two roots are negative. Thus, for any choice of parameters, $p(x_1)$ has exactly one positive root.

Now that we know there is a unique positive equilibrium point for any set of parameters, the next obvious step is to investigate its asymptotic stability. Our initial approach was to perform simulations in MATLAB.

1. First, we have generated two random arrays:
 - a. Array $R1$ of size $n \times 15$, where n can be any positive integer. Each row of $R1$ corresponds to a vector of model parameters $\mathbf{p} = [\alpha_1, \alpha_2, \alpha_3, \alpha_4, \alpha_5, \alpha_6, \gamma_2, \gamma_3, \gamma_4, \gamma_5, \gamma_h, k_{52}, k_{53}, k_{54}, k_{15}]$.
 - b. Array $R2$ of size $n \times 2$. Each row of array $R2$ corresponds to a vector $[Fe_{ex}, Hep]$.
 - c. We set $n = 10,000,000$.

2. $R1$ was generated using an *exponential distribution* with mean $\mu = 1000$. $R2$ was generated using a *continuous uniform distribution* with lower endpoint 0 and upper endpoint 1.
3. Next, we have initiated three output files that recorded:
 - a. parameters,
 - b. steady state (equilibrium point), and
 - c. an eigenvalue of the Jacobian matrix with maximum real part for that set of parameters.
4. For $i = 1 : n$
 - a. Each parameter $\sigma_j \in \mathbf{p}$ was assigned $R1(i, j)$. Fe_{ex} and Hep were assigned $R2(i, 1)$ and $R2(i, 2)$, respectively.
 - b. For each i , the steady state (equilibrium point), Jacobian matrix and eigenvalues of the Jacobian matrix were computed.
 - c. Parameters, steady state and an eigenvalue of the Jacobian matrix with maximum real part were recorded.
5. An array that contained an eigenvalue with maximum real part returned:

$$\min = -2.9967 \times 10^3$$

$$\max = -5.2392 \times 10^{-8}.$$

In addition, we have carried out another simulation in which each parameter was randomly chosen from a continuous uniform distribution with lower endpoint 0 and upper endpoint 10,000. We did 10,000,000 runs and have recorded eigenvalues with maximum real part in the interval $(-8.5196 \times 10^3, -1.3081 \times 10^{-6})$.

This simulation suggests that the positive equilibrium point might be asymptotically stable in general. It is worth pointing out that the simulation returned some complex eigenvalues, but all of them with negative real parts. This implies the possibility of *decaying* oscillatory solutions. However, our study also suggests that System (1) does not have limit cycle oscillations, and we hope to provide a mathematical proof of asymptotic stability in the near future.

3.4. Identifiability—As our work progresses and as we collect more experimental data, the next obvious step would be estimation of parameters. This brings us to the question of whether or not the parameters are identifiable from measured output data and, if not, whether we can find combinations of parameters that we can use to reparameterize the system? Thus, before we invest time in experiments and begin estimation of parameters we must find out which of our parameters can be uniquely recovered under ideal conditions in order to maximize the predictive power of our model. In other words, we do not want two different sets of parameters to lead to the same dynamics. To summarize, System (1) is *identifiable* if model parameters $\mathbf{p} = [\alpha_1, \alpha_2, \alpha_3, \alpha_4, \alpha_5, \alpha_6, \gamma_2, \gamma_3, \gamma_4, \gamma_5, \gamma_h, k_{52}, k_{53}, k_{54}, k_{15}]$ can be uniquely determined from measurable output data, otherwise it is *unidentifiable*.

We use a differential algebra approach to answer this identifiability question for our ODE system. There are several reasons for our choice of methodology: (i) identifiability of nonlinear systems can be cumbersome, (ii) System (1) can be written as a system of differential polynomials, therefore we can apply the differential algebra framework, which is well defined and established, and (iii) differential algebra computational algorithms have been implemented in the software package MAPLE, which enables easy computation. The

details behind the theory of the algorithms and terminology of differential algebra approach are beyond the scope of this paper, hence we only briefly touch on the subject and give a somewhat informal sketch of steps required to answer the identifiability question for our system. For more details the reader is encouraged to consult [27, 28, 29, 30, 31, 32, 33]. We adopt notation and definitions similar to those in [28, 32].

System (1) can be written in the following form:

$$\begin{aligned}\dot{\mathbf{x}}(t, \mathbf{p}) - \mathbf{f}(\mathbf{x}(t, \mathbf{p}), t; \mathbf{p}) &= \mathbf{0}, \\ \mathbf{y}(t, \mathbf{p}) - \mathbf{g}(\mathbf{x}(t, \mathbf{p}), \mathbf{p}) &= \mathbf{0}.\end{aligned}\tag{4}$$

Here,

$\mathbf{x}$ is a state vector representing concentrations,

$\mathbf{y}$ is an output vector representing time histories of concentrations,

$\mathbf{p}$ is a parameter vector, $\mathbf{p} > 0$,

$\mathbf{f}$ and $\mathbf{g}$ are rational polynomial functions.

Note that by adding, subtracting, multiplying, or differentiating both sides of (4), we can produce new equations. Thus, the next step is to transform system (4) into an equivalent system in which one or more equations have the state variable $\mathbf{x}$ eliminated. We use the *diffalg* MAPLE package to compute an equivalent system. This package provides a differential elimination algorithm for systems of algebraic differential equations and is based on the theory of Ritt and Kolchin [30, 31]. The basic idea behind this algorithm is to perform successive substitutions in order to eliminate state variables so that one or more equations are only in terms of the measurable output $\mathbf{y}$ and the parameters $\mathbf{p}$. The resulting set of differential equations will be a *minimal* set, called a *characteristic set*. Characteristic set contains the same information as the original system, but because of its special structure, it allows testing of parameter identifiability without difficulty.

Equations of interest in the characteristic set need not be monic, that is, the coefficient of a leading monomial need not be 1. Thus to fix coefficients uniquely one must normalize these equations [33]. Each normalized equation, which is independent of the state variable $\mathbf{x}$, will be of the form $\sum c_j(\mathbf{p})\phi_j(\mathbf{y})$, where the coefficients $c_j(\mathbf{p})$ are rational functions in parameters $\mathbf{p}$, and the $\phi_j(\mathbf{y})$ are monomials in $\mathbf{y}, \dot{\mathbf{y}}, \ddot{\mathbf{y}}, \dots$ (the derivatives are with respect to time). By construction, the $\phi_j(\mathbf{y})$ are linearly independent, hence if $\mathbf{p}^*$ is any arbitrary point in the parameter space, then

$$\sum c_j(\mathbf{p})\phi_j(\mathbf{y}) = \sum c_j(\mathbf{p}^*)\phi_j(\mathbf{y}) \text{ if and only if } c_j(\mathbf{p}) = c_j(\mathbf{p}^*) \text{ for all } j.$$

In particular, checking global identifiability of our system translates into checking the injectivity of the map $\mathbf{c}(\mathbf{p})$ [27, 28, 32]. Informally speaking, we must solve a system of algebraic equations $\mathbf{c}(\mathbf{p}) - \mathbf{c}(\mathbf{p}^*) = \mathbf{0}$ for $\mathbf{p}$. One can solve $\mathbf{c}(\mathbf{p}) - \mathbf{c}(\mathbf{p}^*) = \mathbf{0}$ using standard computer algebra algorithms. These equations are called an *exhaustive summary* [34].

If there is a unique solution $\mathbf{p} = \mathbf{p}^*$, then the system (4) is said to be *a priori globally identifiable*. If there are finitely many distinct solutions then it is *locally identifiable*, and for infinitely many solution, it is *unidentifiable*. In the case of unidentifiability sometimes it is possible to find identifiable combinations by simple inspection, as the reader will see in our case, but for more complicated systems Meshkat et al. [28] have developed an algorithm that finds globally identifiable parameter combinations for nonlinear ODE systems.

We now carry out the steps sketched above for our system. Recall, $x_1 = [\text{LIP}]$, $x_2 = [\text{TfR1}]$, $x_3 = [\text{Fpn}]$, $x_4 = [\text{Ft}]$, and $x_5 = [\text{Active IRP's}]$. We rewrite ODE system (1) in the form (4) by clearing denominators:

$$\begin{aligned}\dot{x}_1(k_{54}+x_5) - ((k_{54}+x_5)(\alpha_1 Fe_{ex}x_2 + \gamma_4 x_4 - \alpha_6 x_1 x_3) - \alpha_4 x_1 k_{54}) &= 0, \\ \dot{x}_2(k_{52}+x_5) - (\alpha_2 x_5 - \gamma_2 x_2(k_{52}+x_5)) &= 0, \\ \dot{x}_3(k_{53}+x_5) - (\alpha_3 k_{53} - (k_{53}+x_5)(\gamma_3 + \gamma_h Hep)x_3) &= 0, \\ \dot{x}_4(k_{54}+x_5) - (\alpha_4 x_1 k_{54} - (k_{54}+x_5)\gamma_4 x_4) &= 0, \\ \dot{x}_5(k_{15}+x_1) - (\alpha_5 k_{15} - (k_{15}+x_1)\gamma_5 x_5) &= 0, \\ y_1 - x_1 = 0, y_2 - x_2 = 0, y_3 - x_3 = 0, y_4 - x_4 = 0, y_5 - x_5 = 0.\end{aligned}\tag{5}$$

We assume that $\mathbf{y} = \mathbf{x}$ as output function, where $y_i = x_i$ for all $i \in \{1, \dots, 5\}$. That is, we assume that we can measure all the molecular species in the model. The transformed system is (we only list equations that are in terms of output vector $\mathbf{y}$ and parameters $\mathbf{p}$):

$$\begin{aligned}k_{54}\dot{y}_1 + \dot{y}_1 y_5 - \gamma_4 y_5 y_4 + \alpha_6 y_5 y_3 y_1 - \alpha_1 Fe_{ex} y_5 y_2 - \gamma_4 k_{54} y_4 + \alpha_6 k_{54} y_3 y_1 - \alpha_1 Fe_{ex} k_{54} y_2 + \alpha_4 k_{54} y_1 &= 0, \\ -k_{52}\dot{y}_2 - \dot{y}_2 y_5 + \alpha_2 y_5 - \gamma_2 k_{52} y_2 - \gamma_2 y_5 y_2 &= 0, \\ -k_{53}\dot{y}_3 - \dot{y}_3 y_5 - (\gamma_3 + \gamma_h Hep)k_{53} y_3 - (\gamma_3 + \gamma_h Hep)y_5 y_3 + \alpha_3 k_{53} &= 0, \\ k_{54}\dot{y}_4 + \dot{y}_4 y_5 + \gamma_4 k_{54} y_4 + \gamma_4 y_5 y_4 - \alpha_4 k_{54} y_1 &= 0, \\ -k_{15}\dot{y}_5 - \dot{y}_5 y_1 - \gamma_5 k_{15} y_5 - \gamma_5 y_5 y_1 - \alpha_5 k_{15} &= 0.\end{aligned}\tag{6}$$

Recall that Fe_{ex} and Hep serve as fixed control parameters. Let $\mathbf{p}^*$ be an arbitrary vector in the parameter space, $\mathbf{p}^* = [\alpha_1^*, \alpha_2^*, \alpha_3^*, \alpha_4^*, \alpha_5^*, \alpha_6^*, \gamma_2^*, \gamma_3^*, \gamma_4^*, \gamma_5^*, \gamma_h^*, k_{52}^*, k_{53}^*, k_{54}^*, k_{15}^*]$. We list the *exhaustive summary*:

$$\begin{aligned}k_{54} - k_{54}^* &= 0, & \gamma_4 - \gamma_4^* &= 0, & \alpha_6 - \alpha_6^* &= 0, & \gamma_4 k_{54} - \gamma_4^* k_{54}^* &= 0, \\ \alpha_4 k_{54} - \alpha_4^* k_{54}^* &= 0, & \alpha_2 - \alpha_2^* &= 0, & k_{52} - k_{52}^* &= 0, & \alpha_6 k_{54} - \alpha_6^* k_{54}^* &= 0, \\ \gamma_2 k_{52} - \gamma_2^* k_{52}^* &= 0, & \gamma_2 - \gamma_2^* &= 0, & k_{53} - k_{53}^* &= 0, & \alpha_3 k_{53} - \alpha_3^* k_{53}^* &= 0, \\ \gamma_5 k_{15} - \gamma_5^* k_{15}^* &= 0, & k_{15} - k_{15}^* &= 0, & \gamma_5 - \gamma_5^* &= 0, & \alpha_5 k_{15} - \alpha_5^* k_{15}^* &= 0,\end{aligned}$$

$$\begin{aligned}\alpha_1 Fe_{ex} - \alpha_1^* Fe_{ex} &= 0, & (\gamma_3 + \gamma_h Hep) - (\gamma_3^* + \gamma_h^* Hep) &= 0, \\ \alpha_1 Fe_{ex} k_{54} - \alpha_1^* Fe_{ex} k_{54}^* &= 0, & (\gamma_3 + \gamma_h Hep)k_{53} - (\gamma_3^* + \gamma_h^* Hep)k_{53}^* &= 0.\end{aligned}$$

Our system is simple enough to check for injectivity by inspection. One can easily see that all parameters will be identifiable except for γ_3 and γ_h , that is, we cannot find a unique solution to γ_3 and γ_h in terms of $\mathbf{p}^*$ only. These two parameters depend on each other and lead to infinitely many solutions. This implies that our system is unidentifiable. We can reparameterize our system into an identifiable one by letting $\gamma = \gamma_3 + \gamma_h Hep$. In our future studies, we will use the reparameterized system for parameter estimation and estimation of γ to investigate biologically plausible conditions for self-degradation of ferroportin and the degradation of ferroportin induced by hepcidin at the various levels of the control parameter Hep .

4. Model Validation

In macrophages, it has been shown that increases in ferroportin result in lowered levels of ferritin [35]. Experiments have also shown that this mechanism is preserved in breast cancer cells [3], suggesting that it is a conserved feature of iron regulation. To test the predictive power of our model, we have investigated the existence of the same mechanism for a non-

tumorigenic epithelial cell line, MCF 10A. This is the type of cell to which the model applies. As shown below, the mechanism is indeed predicted by the model for any choice of parameters, and the experiment did reveal an inverse relationship between ferroportin and ferritin levels as well as an increase in IRP2 (Figure 2).

4.1. Methods—The experimental protocol for model validation was similar to that described previously in [3]. Briefly, the MCF10A human mammary epithelial cells were cultured in DMEM-F12 supplemented with 5% horse serum, and 1% penicillin-streptomycin, 10 µg/ml insulin, 20 ng/ml EGF, 0.5 mg/ml Hydrocortisone, 100 ng/ml Cholera Toxin (all from Gibco/Invitrogen, Carlsbad, CA) at 37 °C in a humidified atmosphere containing 5% carbon dioxide. Because MCF10A cells are difficult to transfect, we used the lentivirus-mediated method to overexpress ferroportin in MCF10A cells. An expression vector encoding a functional Ferroportin-GFP fusion protein was obtained as a generous gift from Dr. Jerry Kaplan (University of Utah)[36]. The Ferroportin-GFP cassette was amplified by PCR and subcloned into a lentiviral vector carrying a puromycin resistance marker (gift of Guangchao Sui, Wake Forest School of Medicine). Lentivirus particles were produced by transient co-transfection of the Fpn expression vector and packaging vectors (VSVG, pMDLG, and RSV-REV) into 293T cells [37, 38]. Viral particles containing a control empty vector were prepared similarly. Lentivirus was harvested after 48 hours and used to infect the MCF10A cell line. The infection efficiencies were over 90% based on the EGFP fluorescence. The cells were collected for subsequent analysis at 48 hours after infection.

4.1.1. Western Blotting: Western blotting was used to assess levels of ferritin and IRP2. Sub-confluent cells were washed in phosphate-buffered saline (PBS) and scraped. Whole cellular protein was extracted with NP-40 lysis buffer (25mM Tris pH 7.4, 1% Triton X100, 1% SDS, 1% sodium deoxycholate, 150 mM NaCl, 2 µg/ml aprotinin, 1mM PMSF) containing complete protease inhibitor cocktail (Roche Diagnostics). Total protein concentrations were determined using BCA reagent (Pierce). Protein samples (10–50 µg) were separated on 12% gels (SDS-PAGE) and transferred on PVDF membrane (Millipore). Equal loading and transfer were confirmed by staining the membrane with Ponceau S, as well as incubation with anti-GAPDH antibody (Fitzgerald, Cat #RDI-TRK5G4-6C5, Concord, MA) followed by goat anti-mouse antibody (Bio-Rad, Cat #170-6516, Hercules, CA). Ferritin H was detected as previously described [39]. IRP2 was detected with the antibody Sc-33682 (Santa Cruz Biotechnology). Supersignal West Pico Chemiluminescent substrate was used for detection (Pierce).

4.1.2. Quantification of Western Blots: Quantification of blot intensity was performed in *ImageJ* [40] by subtracting background and measuring the integrated density of each band. After normalization to GAPDH, quantification revealed an approximate increase from 1 to 1.6 in IRP2 and a decrease from 1 to 0.1 in H ferritin relative to a vector.

4.2. Theoretical Approach to Model Validation—We prove that our model predicts the experiment, that is, for any set of parameters we show that overexpressing ferroportin results in the decrease of ferritin and an increase in IRP's at steady state.

Denote system (1) by $\dot{\mathbf{x}} = \mathbf{f}(\mathbf{x})$. To represent overexpression of ferroportin we multiply the production rate of ferroportin, α_3 , by a factor $w > 1$, i.e.

$$\dot{x}_3 = w\alpha_3 \frac{k_{53}}{k_{53} + x_5} - (\gamma_3 + \gamma_h \text{Hep})x_3.$$

We call the new system $\dot{\mathbf{x}}_w = \mathbf{f}_w(\mathbf{x})$. Recall that solving system $\dot{\mathbf{x}}$ for an equilibrium resulted in a single cubic polynomial in terms of $x_1 = [\text{LIP}]$, see Polynomial (3). Notice that the coefficient of x_1 contains both negative and positive parts. We write $c = c^+ - c^-$, where

$$\begin{aligned} c^+ &= \gamma_5 k_{15} k_{53} \alpha_3 \alpha_6 \gamma_2 k_{15} (\alpha_5 + \gamma_5 k_{52}), \\ c^- &= \gamma_5 k_{15} k_{53} \alpha_1 \alpha_2 \alpha_5 Fe_{ex}(\gamma_3 + \gamma_h Hep), \end{aligned}$$

and write

$$p(x_1) = ax_1^3 + bx_1^2 + (c^+ - c^-)x_1 + d. \quad (7)$$

Solving system $\dot{\mathbf{x}}_w$ for an equilibrium results in a very similar polynomial in terms of $x_1 = [\text{LIP}]$:

$$p_w(x_1) = wax_1^3 + wbx_1^2 + (wc^+ - c^-)x_1 + d. \quad (8)$$

Remark 3. Since $w > 1$, the sign of the coefficients in p_w is the same as for the coefficients in p , that is, $wa > 0$, $wb > 0$ and $d < 0$, while the coefficient $wc^+ - c^-$ of the linear term could be of either sign. Also, recall that the proof of Corollary 2 does not depend on the sign of the linear term. Thus, by an argument similar to one in Corollary 2 we conclude that p_w has exactly one positive root, and the other two roots are either negative or complex.

Lemma 4. Let p and p_w be two polynomials as described by Equations (7) and (8), and let $x_1 > 0$ and $x_{1w} > 0$ be positive roots of p and p_w , respectively. Then $x_{1w} < x_1$ for all $w > 1$, that is, an overexpression of ferroportin results in a decrease of LIP at the steady state.

Proof. Let $x_1 > 0$ and $x_{1w} > 0$ be positive roots of p and p_w . Then $p(x_1) = 0$ and $p_w(x_{1w}) = 0$. Note that p and p_w have the same y -intercept at d . Also, p and p_w intersect in the following points:

$$x=0, \quad x = -k_{15}, \quad \text{and} \quad x = \frac{-(\alpha_5 k_{15} + \gamma_5 k_{15} k_{52})}{\gamma_5 k_{52}}.$$

This means that p and p_w have no intersection in $(0, \infty)$. But,

$$\begin{aligned} p_w(x_1) &= wax_1^3 + wbx_1^2 + (wc^+ - c^-)x_1 \\ &\quad - c^-)x_1 \\ &\quad + d = w(ax_1^3 \\ &\quad + bx_1^2 + (c^+ - c^-)x_1 \\ &\quad + d) + wc^-x_1 - c^-x_1 + d - dw = w(p(x_1)) + (w - 1)(c^-x_1 \\ &\quad - d) = w \cdot 0 \\ &\quad + (w - 1)(c^-x_1 \\ &\quad - d) = (w - 1)(c^-x_1 - d). \end{aligned}$$

Since, $w > 1$, $c^- > 0$, and $d < 0$, we have that $p_w(x_1) > 0$. This implies that p_w will cross the x -axis before p , i.e., $x_{1w} < x_1$.

Let $(x_1, x_2, x_3, x_4, x_5)$ and $(x_{1w}, x_{2w}, x_{3w}, x_{4w}, x_{5w})$ represent steady states (equilibrium points) of systems $\mathbf{x}$ and $\mathbf{x}_w$, respectively. Note that x_1 and x_{1w} are positive roots of p and p_w . We write the steady state equations of $\mathbf{x}$ and $\mathbf{x}_w$ in terms of x_1 and x_{1w} .

Steady state of $\mathbf{x}$:

$$\begin{aligned} x_2 &= \frac{\alpha_2 \alpha_5 k_{15}}{\gamma_2 (\alpha_5 k_{15} + \gamma_5 k_{52} (k_{15} + x_1))}, \\ x_3 &= \frac{\alpha_3 \gamma_5 k_{53} (k_{15} + x_1)}{(\gamma_3 + \gamma_h \text{Hep}) (\alpha_5 k_{15} + \gamma_5 k_{53} (k_{15} + x_1))}, \\ x_4 &= \frac{\alpha_4 \gamma_5 k_{54} x_1 (k_{15} + x_1)}{\gamma_4 (\alpha_5 k_{15} + \gamma_5 k_{54} (k_{15} + x_1))}, \\ x_5 &= \frac{\alpha_5 k_{15}}{\gamma_5 (k_{15} + x_1)}. \end{aligned} \quad (9)$$

Steady state of $\mathbf{x}_w$:

$$\begin{aligned} x_{2w} &= \frac{\alpha_2 \alpha_5 k_{15}}{\gamma_2 (\alpha_5 k_{15} + \gamma_5 k_{52} (k_{15} + x_{1w}))}, \\ x_{3w} &= \frac{w \alpha_3 \gamma_5 k_{53} (k_{15} + x_{1w})}{(\gamma_3 + \gamma_h \text{Hep}) (\alpha_5 k_{15} + \gamma_5 k_{53} (k_{15} + x_{1w}))}, \\ x_{4w} &= \frac{\alpha_4 \gamma_5 k_{54} x_{1w} (k_{15} + x_{1w})}{\gamma_4 (\alpha_5 k_{15} + \gamma_5 k_{54} (k_{15} + x_{1w}))}, \\ x_{5w} &= \frac{\alpha_5 k_{15}}{\gamma_5 (k_{15} + x_{1w})}. \end{aligned} \quad (10)$$

Observe that, since $x_{1w} < x_1$, it is not obvious from the steady-state equations that multiplying the production rate of Fpn by a factor $w > 1$ corresponds to overexpression of Fpn, i.e., that $x_{3w} > x_3$ for all $w > 1$.

Lemma 5. *Multiplying the production rate of Fpn by a factor $w > 1$ corresponds to overexpression of Fpn.*

Proof. We need to show that at the steady state and for an arbitrary set of parameters, $x_{3w} > x_3$ for all $w > 1$. Since both x_3 and x_{3w} are strictly positive, in particular $x_3 \neq 0$, we have

$$\frac{x_{3w}}{x_3} = \frac{w(\alpha_5 k_{15} + \gamma_5 k_{53} (k_{15} + x_1)) (k_{15} + x_{1w})}{(k_{15} + x_1) (\alpha_5 k_{15} + \gamma_5 k_{53} (k_{15} + x_{1w}))} > \frac{w(\alpha_5 k_{15} + \gamma_5 k_{53} (k_{15} + x_1)) (k_{15} + x_{1w})}{(k_{15} + x_1) (\alpha_5 k_{15} + \gamma_5 k_{53} (k_{15} + x_1))} = \frac{w(k_{15} + x_{1w})}{(k_{15} + x_1)}.$$

Note that x_1 and x_{1w} are positive roots of p and p_w , respectively, i.e., $p(x_1) = 0$ and $p_w(x_{1w}) = 0$.

To show that $\frac{w(k_{15} + x_{1w})}{(k_{15} + x_1)}$ is greater than 1 we need to show that $x_1 < w x_{1w}$. Consider a polynomial p_o with the same behavior as p and p_w defined as follows,

$$p_o(x) = ax^3 + bx^2 + (wc^+ - c^-)x + wd.$$

The only intersection between p and p_o is $x = -\frac{d}{c^+}$. Since $d < 0$ and $c^+ > 0$ this intersection occurs on $(0, \infty)$, where

$$-\frac{d}{c^+} = \frac{\alpha_1 \alpha_2 \alpha_5 \text{Fe} (\gamma_3 + \gamma_h \text{Hep}) (\alpha_5 + \gamma_5 k_{53})}{\alpha_3 \alpha_6 \gamma_2 \gamma_5 (\alpha_5 + \gamma_5 k_{52}) k_{53}}.$$

Now, $p(-\frac{d}{c^+}) = p_o(-\frac{d}{c^+}) = fg$, where

$$f = \frac{(\alpha_1 \alpha_2 \alpha_5 Fe_{ex})^2 (\gamma_3 + \gamma_h Hep)^2 (\alpha_5 + \gamma_5 k_{53})}{(\alpha_3 \alpha_6 \gamma_2 k_{53})^2 \gamma_5 (\alpha_5 + \gamma_5 k_{52})^3},$$

$$g = \alpha_1 \alpha_2 \alpha_5 Fe_{ex} (\gamma_3 + \gamma_h Hep) k_{52} (\alpha_5 + \gamma_5 k_{53})^2 + \alpha_3 \alpha_6 \gamma_2 k_{15} (\alpha_5 + \gamma_5 k_{52}) k_{53} (\alpha_5^2 + 2\alpha_5 \gamma_5 k_{52} + \gamma_5^2 k_{52} k_{53}).$$

Notice that f and g are strictly positive, hence

$$p\left(-\frac{d}{c^+}\right) = p_o\left(-\frac{d}{c^+}\right) > 0.$$

This means that p and p_o intersect above the x -axis. In addition, the y -intercept of p_o is at $wd < d$. Therefore, if r is the positive root of p_o , then $x_1 < r$. But,

$$\begin{aligned} p_o(wx_{1w}) &= a(wx_{1w})^3 \\ &+ b(wx_{1w})^2 \\ &+ (wc^+ - c^-)(wx_{1w}) \\ &+ wd = a(wx_{1w})^3 \\ &+ b(wx_{1w})^2 \\ &+ (wc^+ - c^-)(wx_{1w}) \\ &+ wd - (wp_w(x_{1w})) \\ &= a(w - 1)w^2 x_{1w}^3 > 0. \end{aligned}$$

Then $x_1 < r < wx_{1w}$, i.e., $x_1 < wx_{1w}$. This implies that $x_1 < wx_{1w} + k_{15}(w - 1)$. Hence, we conclude that

$$\frac{x_{3w}}{x_3} > \frac{w(k_{15} + x_{1w})}{(k_{15} + x_1)} > 1.$$

Theorem 6 (Validation Theorem). *For an arbitrary choice of parameters in the model and an arbitrary $w > 1$, an overexpression of ferroportin in the model results in a decrease of LIP and Ft and in an increase of TfR1, and active IRP's at the steady state, that is,*

$$x_{1w} < x_1, x_{2w} > x_2, x_{4w} < x_4, \text{ and } x_{5w} > x_5.$$

Proof. By Lemma 4, $x_{1w} < x_1$. Hence,

$$\begin{aligned}
 x_{2w} - x_2 &= \frac{\alpha_2 \alpha_5 \gamma_5 k_{15} k_{52} (x_1 - x_{1w})}{\gamma_2 (\alpha_5 k_{15} + \gamma_5 k_{52} (k_{15} + x_1)) (\alpha_5 k_{15} + \gamma_5 k_{52} (k_{15} + x_{1w}))} > 0, \\
 x_{4w} - x_4 &= \frac{-(\alpha_4 \gamma_5 k_{54} (x_1 - x_{1w})) (\gamma_5 k_{54} (k_{15} + x_1) (k_{15} + x_{1w}) + \alpha_5 k_{15} (k_{15} + x_1 + x_{1w}))}{\gamma_4 (\alpha_5 k_{15} + \gamma_5 k_{54} (k_{15} + x_1)) (\alpha_5 k_{15} + \gamma_5 k_{54} (k_{15} + x_{1w}))} < 0, \\
 x_{5w} - x_5 &= \frac{\alpha_5 k_{15} (x_1 - x_{1w})}{\gamma_5 (k_{15} + x_1) (k_{15} - x_{1w})} > 0.
 \end{aligned}$$

This concludes the proof.

It is worth pointing out that Theorem 6 makes additional predictions that could be tested in the future.

5. Discussion

We have presented a mathematical model of the core control system of intracellular iron homeostasis in breast epithelial cells. The analysis and validation of the model is complicated by the fact that it has 15 parameters whose numerical values are not known for this cell type. The two common ways to deal with unspecified parameters is to either fit the model parameters to available experimental data or to carry out an analysis of the model, either analytically or through simulation, that explores different regions of the parameter space. There are well-known problems with parameter estimation. We decided to do both validation and analysis of model dynamics using a general analysis. Firstly, we show analytically that the model has a unique steady state. Through extensive simulations we show that this steady state is stable. Furthermore, we show that the model is capable of showing damped oscillations. An important consequence of this observation is that the model rules out sustained oscillations of the labile iron pool in particular. While there is some experimental evidence, cited earlier, that intracellular iron levels are tightly constrained, there remains the possibility of oscillatory behavior. Our results provide evidence to the contrary. Another important observation is that these model properties hold independent of the parameters. The interactions incorporated in the model are assumed to be standard Michaelis-Menten kinetics, which is a reasonable assumption. This implies that the model properties are largely inherent in the topology of the network connections.

We have validated the model with experimental data that show that it correctly predicts the effect of perturbing one of the network nodes on the steady state levels of two of the other nodes. Furthermore, we provide hypotheses about the effect on other nodes, which remain to be validated.

There are two obvious next steps to be taken. One is to model the network in somewhat finer detail by separating the two IRPs and by treating the saturation of the iron sequestration protein ferritin more realistically. The other step is to expand the network to include heme synthesis, heme degradation, iron cluster biogenesis and other intracellular processes that require iron [2]. A third step that is eventually necessary and highly relevant for many applications is to include the oxidative stress response network and its interaction with iron metabolism. Finally, it is important to carry out a more extensive validation of the model with experimental data. It is possible to measure several key parameters in the model, another crucial step toward a detailed mechanistic model of intracellular iron metabolism.

An expanded and more detailed model can then be used for the purpose of identifying key regulators in the iron metabolism network that are modified as normal breast epithelial cells transition to malignancy. The model serves as an *in silico* laboratory in which one can introduce modifications to the network that can explain data characterizing the malignant phenotype. Successful modifications then form the basis of further experimental validation.

Highlights

- Iron is essential for cellular metabolism, but can also cause damage. Levels are tightly constrained.
- Focus on core control system in breast epithelial cells. Connection between iron homeostasis and breast cancer
- Construct, analyze, and validate a differential equations model of this control system.

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Figure 1. Regulatory feedback loops in iron metabolism. Species depicted are ferroportin ($[Fpn] = x_3$); hepcidin (Hep); transferrin receptor 1 ($[TfR] = x_2$); ferritin ($[Ft] = x_4$); active iron regulatory proteins 1 and 2 ($[Active\ IRP's] = x_5$). Solid lines indicate positive or negative regulation; dotted lines indicate reactions that consume or produce the indicated species.

Figure 2. Effect of Fpn overexpression in MCF10A cells. Quantification revealed that Fpn overexpression increased IRP2 from 1 to approximately 1.6 and decreased Ferritin H from 1 to 0.1 (see Methods section).