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Robustness in filter designs. Implications for receptor internalization.

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A common signaling processing step is to filter the signal. In biology there are amble examples where the physiological response depends on the duration of the signal. A simple way to obtain such a filter effect is with the help of a delay circuitry. There are at least two principally different ways to obtain the delay: activate an activator or by inhibiting an inhibitor. Previously a model based on the first option has been proposed in connection with the early signaling events in the mammalian response to Insulin. Here we consider the equivalent model based on the second option. We demonstrate that although the two options are able to obtain the exact same filter characteristics, the robustness to external perturbations are very different. In particular is the inhibitory version insensitive to variations in the concentration of the inhibitor, while the cross-over frequency in the activator case depends linearly on the concentration of the activator. The cross-over frequency determines the timescale that separates the two different physiological responses metabolic versus mitogenic. This kind of robustness is thus very important in the design of drugs that interfere with the signaling pathway (Insulin sensitizers). However one can not generally conclude that the inhibitor version is the most robust as in transcriptional feed forward loops the activate an activator version is the most robust. Furthermore we point out that receptor internalization effectively implement a robust delay circuitry, and this raises the intriguing possibility that receptor internalization quite generally is involved in filtering of the external signal.

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I. INTRODUCTION

Living cells often relay a signal from outside the cell to the inside of the cell and nucleus. This signal transduction is carried out by a complex network of molecular transformations and pathways. In some cases it is possible to identify small sub parts of this huge network that act as independent modules, each performing a relative simple task like detecting the rate of change of the concentration of a hormone [1, 2]. One of the simplest examples of such a module is a filter [3] i.e. a module that distinguishes between short transient signals and more sustained signals and only in the later case propagate a signal further down the signal pathway — see Figure 1. A common way to implement a filter is with a delay circuit in which the present signal is compared with the signal a little while ago and only if both are high the signal is passed on [16]. The delaying of the signal is typically obtained either by letting the original signal activate an effector molecule that then activate the output from the filter (if the original signal stays on persistently) or by inhibiting an inhibitor that stop the present signal from propagating downstream. These two methods both give functional filters that, at first sight, are very similar. However as demonstrated below, the robustness and tuneability of these two filter designs are very different. Naturally, this is very important when a drug treatment unadverdently meddle with the concentration of either the inhibitor or activator. As the function of the filter only in some cases depends critically on this concentration. Below this is discussed in more detail in the case of the Insulin receptor.

II. INSULIN

Insulin is a hormone that is involved in diabetes and the regulation of the blood sugar level. However this is not the only physiologic role of Insulin, it also acts as a growth hormone. In the literature these two effects are often broadly

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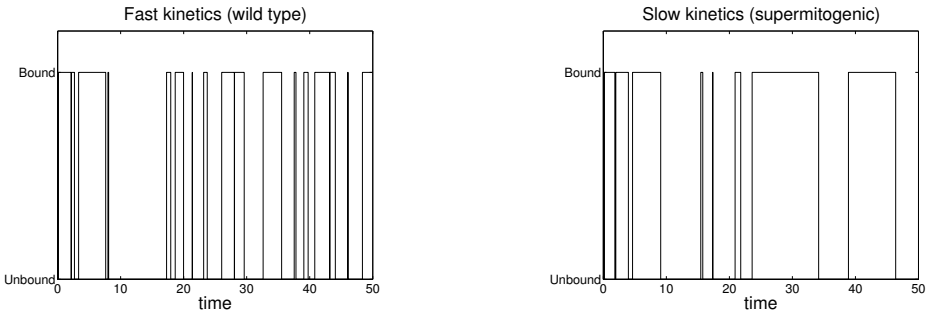

FIG. 1: The binding dynamics of two different Insulin analogues binding to the receptor. On average they are bound the same amount of time (40% of the time) i.e. they have the same affinity, but the dynamics is different. a) wild type Insulin with a typical residence time — the typical length of a peak — of the order minuttes. b) A supermitogenic variant where the residence time can be of order hours. (In the plot it is three times longer than in a)). Only the super mitogenic Insulin (b) is an effective growth hormone, while wild type Insulin (a) mainly acts as a metabolic hormone. Since cells often have many (more than 100) receptors the distinction between the two cases has to be made physically on or by the individual receptor. Thus the filter is localized on the receptor and only in scenario b) a mitogenic signal is passed on downstream.

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referred to as Insulin's metabolic and mitogenic effects, respectively. Here we follow this practice. Experimentally one has established that the mitogenic potency of Insulin depends on the binding kinetics of Insulin towards its receptor. In particular it seems the residence time of Insulin k_- and not the affinity $K_a = k_+/k_-$ is the determining factor [4] — see also figure 1. Insulin analogues with unusual long residence times k_- (and unchanged affinity) are thus supermitogenic and even carcinogenic in some cases [5]. The above experiments [4] furthermore strongly suggests that the filter that gauge k_- is physically located on the individual receptor itself. A time delay circuit model based on the presence of a hitherto unknown mitogenic effector has previously been proposed for this system [6]. Here this model is contrasted with a model where the effector is replaced by a prebound inhibitor that block the Insulin receptor from acting as a growth inducer — see figure 2. A very important characteristic of any filter is the time

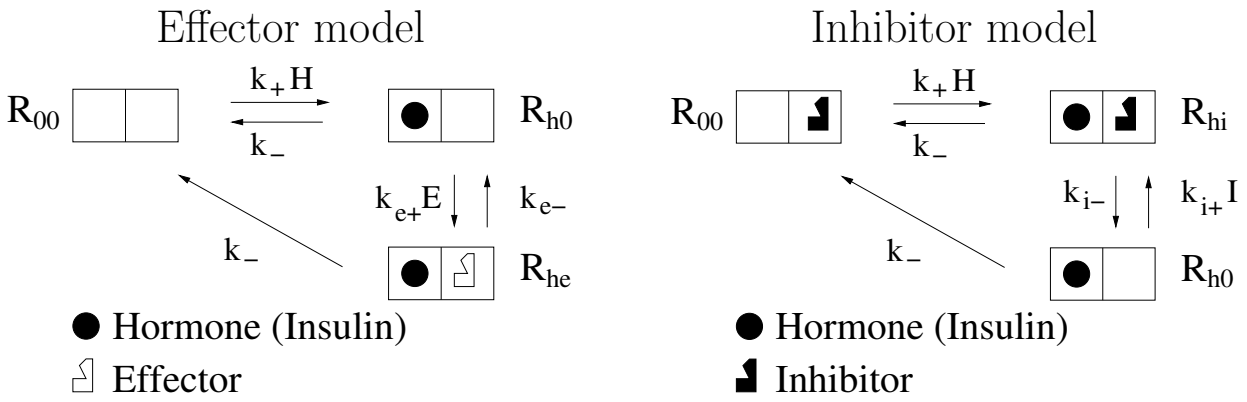

FIG. 2: Effective minimal models for the mitogenic filter in Insulin signaling. Note that the non-equilibrium phosphyrolation of the receptor effectively makes the kinetic parameters independent of each other. In a) the model considered in [6] is depicted. R_{00} is the unbound receptor. R_{h0} is the receptor with the hormone (Insulin represented as a dark spot) bound. However no mitogenic signal is transmitted before the effector molecule E (light polygon) also binds to the receptor. The time delay introduced by the need to bind the effector molecule after Insulin has bound to the receptor is approximately given by the on-rate $k_{e+}E$, where E is the concentration of effector molecules. If the residence time of the hormone Insulin k_- is smaller than this time, the mitogenic signaling complex R_{he} is very rarely formed. The typical crossover frequency of the filter is thus the on-rate $k_{e+}E$ and as such depends on the concentration of effector molecules E. Our slight variation where the effector has been replaced by a prebound inhibitor is depicted in b). R_{0i} is the receptor with the inhibitor prebound (dark polygon), but without Insulin. R_{Hi} is the receptor with Insulin bound. In this case no mitogenic signal is transmitted before the inhibitor is released from the receptor. The typical waiting time for this to happen is the residence time of the inhibitor k_{i-}. As in a) this is also the typical crossover frequency of the filter, but this time it is independent of the number of inhibitors.
Note that in equilibrium the concentration of the signaling complex (R_{he} in a) and R_{h0} in b)) only depends on the affinity of Insulin k_+H/k_-, so that a non-equilibrium transformation of the receptor upon Insulin binding is an essential ingredient in deriving the effective models a) and b).

	Effector		Inhibitor	
Signal strength : $R_{\text{Signaling}}/R_{\text{Hormone bound}}$	$\frac{k_{e+}E}{k_{e+}E+k_{e-}}$	$\frac{k_{e+}E+k_{e-}}{k_{e+}E+k_{e-}+k_{-}}$	$\frac{k_{i-}}{k_{i-}+k_{i+}I}$	$\frac{k_{i-}+k_{i+}I}{k_{i-}+k_{i+}I+k_{-}}$
Maximum ($k_{-}=0$)	$\frac{k_{e+}E}{k_{e+}E+k_{e-}}$		$\frac{k_{i-}}{k_{i-}+k_{i+}I}$	
Cross-over frequency k_c (k_{-} value, where half maximum is obtained)	$k_{e+}E+k_{e-}$		$k_{i-}+k_{i+}I$	
k_c, when the maximum is big (~ 1)	$k_{e+}E$		k_{i-}	

TABLE I: Characteristics of the effective filter in the original model with the delay carried out by an effector molecule (Fig. 2a) and our model variation with a prebound inhibitor (Fig. 2b).
In both cases the signal is proportional to the concentration of receptors with hormone bound on the outside $R_{\text{Hormone bound}} = k_{+}H/(k_{-}+k_{+}H)$, that only depends on the affinity of Insulin $K_a = k_{+}/k_{-}$ and not the residence time k_{-} itself. In the notation of Fig. 2 the signaling molecule in the effector case is $R_{\text{Signaling}} = R_{he}$, while in the inhibitor case it is the hormone bound receptor without inhibitor $R_{\text{Signaling}} = R_{h0}$. In both cases the signal transduction is proportional to a function of the form $k_c/(k_c+k_{-})$, where k_c is the cross-over frequency where the signal is reduced to half of its maximum. In the fourth line k_c in the typical regime is listed for the two models. Notice the maximum (second line) is the equilibrium fraction of bound effector/unbound inhibitor (for the two scenarios, respectively) to a hormone occupied receptor and necessarily smaller than 1.

scale that separates long time scales from short ones. E.g. is a minute a long or short residence time and is such an Insulin analogue supermitogenic? In the original effector model, this cross-over timescale is inversely proportional to the number of effector molecules, while in the prebound inhibitor model the cross-over time is virtually independent of the inhibitor concentration. In table I the characteristics of the two models are contrasted to each other. For more details see appendix A. In the original model it is thus possible to tune individual cells such that Insulin mainly acts as a metabolic or a mitogenic agent merely by changing the concentration of the effector molecule. The downside of this tunablity is that the system is very vulnerable to any changes in the possibly very complex regulatory system determining the effector concentration. Naturally this is something designers of Insulin sensitizers have to keep in mind. Last we also want to point out that a model in which one instead consider the delay to be associated with a conformational change of the receptor is very similar to the inhibitor model and as such also is robust.

III. RECEPTOR INTERNALIZATION

The precise molecular mechanism behind the metabolic mitogenic filter is unknown in the Insulin case. Along with many other receptors the Insulin receptor is internalized upon stimulation [7]. Such an internalization is also an example covered by the above model just with the membrane itself playing the role of the inhibitor. As the typical timescale for internalization is of the same order as the insulin residence time (minutes) [8] (5-15 minutes for the EGF receptor [9]) this is a very intriguing possibility. Especially as it is known that the internalized receptor triggers its own signal cascades [10, 11]. The differences between the signaling events from internalized and membrane bound receptor are thus very good putative candidates for the difference in metabolic versus mitogenic responses to Insulin.

Up to know we have mainly considered the filtering effect of the binding kinetics. However receptor internalization quite generally constitutes a robust time delay (on the order of minutes). It is thus an intriguing possibility that this delay is utilized in a filter. Thus if the physiological response to a hormone signal peak lasting less than a few minutes differs from the response to a constant hormone level, then a putative origin is differences in the signaling events triggered by internalized and membrane bound receptors. In passing we want to mention that in the model of the EGF signaling [2] this is not the mechanism responsible for the high pass filter (slope detector) that emerged as both the timescale for the slope detection was too short and in the model it was assumed that the internalized and membrane bound receptors signaled in the same way.

IV. DISCUSSION

With the early signaling events of Insulin as a case story we have shown that although it in theory is possible to create equivalent signaling cascades by inhibiting an inhibitor or activating an activator the robustness of the resulting

circuitry is very different. In the Insulin case the inhibitor version was the most robust. However this is not generally the case as in transcriptional feed forward loops [12–14] the activator version is the most robust. Curiously enough the activator version is also the most common one [12, 15]. We also pointed out that receptor internalization generally is a robust way to create a time delay in signaling cascades. It is thus possible that receptor internalization is involved in filtering the signal. Differences in the membrane bound and internalized receptor downstream signaling are thus good putative targets to explain physiological differences between short transient signals and more sustained signals.

V. ACKNOWLEDGMENTS

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APPENDIX A: INSULIN MODEL IN MORE DETAIL.

1. The model

Here we consider the microscopic model for mitogen signaling where there is an inhibitor of signaling that can bind to the Insulin receptor on the inside. The model is developed under the following general assumptions:

1. The binding of hormone and inhibitor (effector) can be described classically, with constant on and off rates.
2. Only effects directly related to the binding of hormone and inhibitor (effector) are important
3. The inhibitor bind preferentially to hormone-UNoccupied receptors.
4. Signaling only occurs when a hormone is bound to the receptor and no inhibitor is bound.
5. When hormone stop binding to a receptor the inhibitor rapidly binds to the receptor. The concentration of inhibitor is so large that an inhibitor almost all the time is bound to a receptor without a hormone bound (in equilibrium).
6. After the receptor has undergone non-equilibrium phosphorylation upon binding of the hormone the inhibitor prefers not to bind to the receptor anymore. (The inhibitor is released by hormone binding)

The equilibrium system is most easily visualized by looking at Fig. 3.

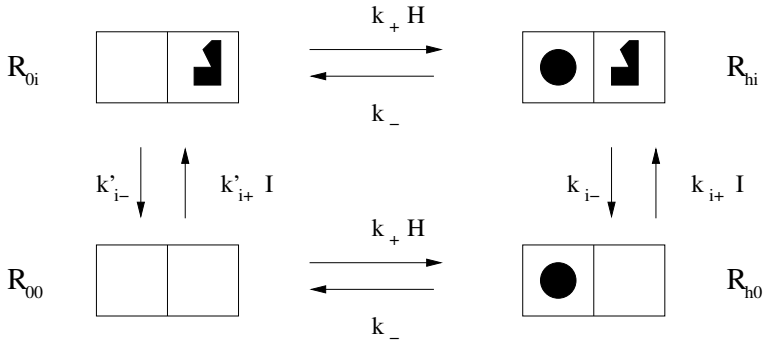

FIG. 3: The inhibitor reaction diagram before the non-equilibrium phosphorylation is considered. Compared with Fig. 2b here the completely unoccupied receptor R_{00} and all reactions are included.

Here R_{00}, R_{h0}, R_{0i}, and R_{hi} are respectively the concentrations of the unoccupied receptor, receptor occupied by hormone only, receptor occupied by inhibitor only, and receptor occupied by both hormone and inhibitor. I is the concentration of inhibitor and H is the concentration of hormone. In Fig. 3 we have made the simplifying assumption that the rates for binding and unbinding of hormone to the receptor (k_+H and k_-, respectively) is independent of its internal configuration (inhibitor bound or not). Assumption 5 above translates into

- The rate for binding of the inhibitor to the unoccupied receptor (R_{00}) is the fastest timescale in the problem i.e. $k'_{I+}I \gg$ all other rates.

2. Non-equilibrium binding kinetics

In fact the system in Fig. 3 is not alone in the world. If it had everything would have been governed by equilibrium dynamics and the different rate constants would have been related to each others (constraints). Furthermore the relative amount of the different kinds of receptors are given by the difference in free energy between them. Hence there is no dependence on the size of the kinetic parameters for the reactions — only their ratios e.g. $\frac{k_+ H}{k_-}$ enter. In particular assumption 3 together with the simplifying assumption that the hormone binding rates are independent of inhibitor binding implies that in equilibrium the inhibitor is also almost always bound to the receptor (It is NOT released from the receptor by hormone binding) However it is known the receptor instantaneously undergoes non-equilibrium phosphorylation upon binding of the hormone. Consequently the rates in Fig. 3 contains an effective non-equilibrium part and we are in practice free to choose the kinetic parameters as we please [6]. In particular we can impose assumption 6 ($k_{i+}I < k_{i-}$) and thus effectively ignore the pathway where the hormone binds to R_{00} in order to produce the signaling complex R_{h0}. With this in mind it is possible with the help of assumption 5 to reduce the dynamics in Fig. 3 to that in Fig. 2b.

a. The rate equations for Fig. 2b

$$\frac{dR_{hi}}{dt} = k_+ H\, R_{0i} - k_- R_{hi} - k_{i-} R_{hi} + k_{i+} I R_{h0} \tag{A1}$$

$$\frac{dR_{h0}}{dt} = k_{i-} R_{hi} - (k_{i+} I + k_-) R_{h0} \tag{A2}$$

The corresponding equations for the effector case (Eq. 1 in [6]) are

$$\frac{dR_{h0}}{dt} = k_+ H\, R_{00} - k_- R_{h0} - k_{e+} E R_{h0} + k_{e-} R_{he} \tag{A3}$$

$$\frac{dR_{he}}{dt} = k_{e+} E R_{h0} - (k_{e-} + k_-) R_{he}\,. \tag{A4}$$

The two set of equations are thus identical upon toggling 0 and i; e and i; association and dissociation on the inside of the receptor. This is also clear directly from the figures. In tabular form this is

inhibitor	effector
R_{0i}	R_{00}
R_{hi}	R_{h0}
R_{h0}	R_{he}
k_{i-}	$k_{e+}E$
k_{i+}	k_{e-}

The mitogenic signal in the inhibitor case is proportional to the steady state fraction of time, where the receptor binds a hormone and no inhibitor R_{h0}. Solving the equations yield (Compare with Eq. 2 in [6])

$$\frac{R_{h0}}{R_{0i} + R_{hi} + R_{h0}} = \frac{k_+ H}{k_- + k_+ H} \frac{k_{i-}}{k_{i-} + k_{i+} I + k_-} \tag{A5}$$

The first factor is just the fraction of time, where hormone is bound to the receptor on the outside $R_{\text{Hormone bound}}$. This is the same in the two scenarios (inhibitor and effector respectively).

3. Differences

We here consider the natural limits where the inhibitor prefers not to be bound to the hormone occupied receptor, while the effector prefers to be bound to the hormone occupied receptor. In formula language we have

$$k_{i-} \gg k_{i+} I \qquad \text{inhibitor} \tag{A6}$$

$$k_{e+} E \gg k_{e-} \qquad \text{effector} \tag{A7}$$

In both these cases the maximum signaling (for $k_- = 0$) is close to its theoretical maximum 1 and relatively independent of the concentration of the signaling molecule I and E respectively. The major difference is in the cross-over frequency

$$k_c \approx k_{i-} \qquad \text{inhibitor} \tag{A8}$$

$$k_c \approx k_{e+}E \qquad \text{effector} \tag{A9}$$

4. Significance of the cross over frequency k_c

Since the maximum is anyway close to one it is only the ratio of k_- to k_c i.e. k_-/k_c that determines the mitogenic potency relative to the metabolic potency. Experimentally this ratio has already been changed by producing Insulin

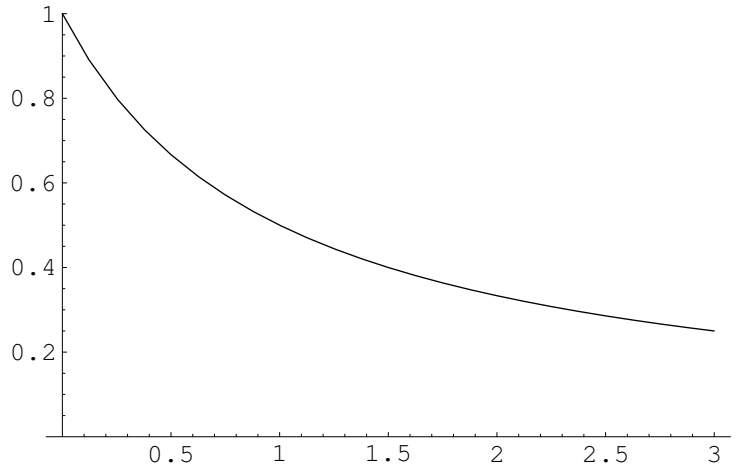

FIG. 4: The filter function $k_c/(k_c + k_-)$ versus k_-/k_c.

analogues with a wide range of values for k_-. One interesting result is that an Insulin analogue with an extremely long residence time (small k_-) was able to induce cancer in rat [5]. In principle standard Insulin could be tuned into the same regime (closer to) by instead increasing k_c. The tuneability of k_c is thus a question of practical interest. Notice also that the experiments with the different Insulin analogues can be used to measure k_c.

a. The inhibitor case

Here $k_c \approx k_{i-}$ so the crossover rate is hardwired into the amino acid sequence and it is hard to change. You have to either use alternative splicing or different post transcriptional changes. It is thus not completely ruled out that the function of the receptor can be tuned during for instance development or from cell type to cell type, but only in a step function like manner where there is one or two different modes of operation. Also Insulin sensitizers and other drugs do not easily change k_c.

b. The effector case

Here $k_c \approx k_{e+}E$ is proportional to the concentration of effector molecules and is thus highly tuneable and can easily vary continuously from cell type to cell type and during development. Since $k_c \approx k_{e+}E$ is an important quantity it is highly likely E is controlled through a delicate feedback mechanism. Drugs designed to alter the downstream signaling processing of the Insulin receptor potentially risk unadverdently to change the level of E and thus the mitogenic potency of Insulin. The worst case scenario is to make Insulin carcinogenic [5].

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[16] This basic design is then in real life often improved by extra control and feedback loops.