Logical analysis of timing-dependent receptor signalling specificity: application to the insulin receptor metabolic and mitogenic signalling pathways

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We present a method for logical analysis of signal-transduction networks, focusing on metabolic and mitogenic signalling by the insulin receptor, with specific emphasis on dependence of the signalling properties on the timing of binding events. We discuss a basic model which demonstrates this dependence (hormone binding leads to activation of the receptor which can lead to a commitment to mitogenic signalling), and show how residence time of the hormone on the receptor can determine the specificity

of signalling between the alternative metabolic or mitogenic pathways. The method gives conditions for the selection of specific branches in the signalling pathway expressed in terms of inequalities among the characteristic activation or deactivation times of components of that pathway. In this way, the conditions for mitogenic signalling can be given in terms of a required range of values of the hormone residence time on the receptor, which is directly related to the kinetic dissociation rate.

INTRODUCTION

Signalling by hormones through their receptors proceeds through complex cascades of interactions which can result in multiple biological effects. For example, insulin, acting through its receptor, triggers a number of biological responses which can be divided into two broad categories. Metabolic processes, associated with uptake and subsequent storage of glucose, are generally taken to be the primary target of insulin receptor signalling, but mitogenic processes associated with DNA replication, cell growth and division are also activated to a lesser or greater degree (for reviews, see [1–3]). Both kinds of signalling begin with hormone binding, which results in receptor autophosphorylation and activation of the receptor tyrosine kinase, followed by a cascade of activation of intracellular signalling molecules (Scheme 1) [4,5]. Early events in the signalling cascade are shared by the metabolic and mitogenic signalling pathways, such as hormone binding and perhaps the binding of the tyrosine-phosphorylated insulin receptor by insulin receptor substrate 1 (IRS-1) [4], IRS-2 [6–8] or Shc [4,9]. Moreover, many components of the known insulin receptor signalling pathways are shared by the predominantly mitogenic insulin-like growth factor I receptor [10–13]; for review, see [2]. At some point along the various intracellular activation pathways, the signal diverges and the intracellular machinery for metabolic or mitogenic response is mobilized. The details of the divergence are not yet well understood, but a number of lines of evidence implicate the receptor itself (for a review, see De Meyts [14]). The interactions of certain branches of the signalling cascade are also becoming clearer. There is accumulating evidence (reviewed in [5]) that the activation of the mitogen-activated protein kinase (MAPK) pathway by the insulin receptor may not be responsible for the metabolic actions of insulin [15,16], whereas phosphatidylinositol 3-kinase appears to play a role in glucose transport, through the stimu-

lation of translocation of GLUT4 transporters to the cell surface [16–18].

The specificity of the signalling can be altered in different ways. Although the predominant signalling mode of the insulin receptor is for metabolic function, there are examples of cell lines with modified specificity of insulin receptor signalling. The growth of the LB strain of T-cell lymphoma cells is strongly stimulated by insulin, and since these cells lack insulin-like growth factor I receptors [19], mitogenic signalling is presumed to occur via the insulin receptor [19, 20]. These cells evidently have an insulin receptor signalling pathway which is biased toward mitogenic activation, implying that a site of selectivity for metabolic versus mitogenic signalling exists somewhere in the chain of signalling events, and that the LB cells may possess a mutation or mutations that are responsible for this altered selectivity. That is, the source of the specificity is within the cells themselves.

There is evidence, however, that the signalling specificity of the insulin receptor can be shifted even in normal cells. Studies with a number of insulin analogues have revealed several that have an increased mitogenic potency in relation to their ability for metabolic stimulation [21–23]. One analogue, with a mutation of His^{B10} → Asp, has been shown to induce mammary tumours in laboratory rats after long-term treatment [24]. A characteristic of all of these analogues is that those with the highest mitogenic potency tend to have the lowest dissociation rates [3,22,25]. That is, the signalling specificity of the interaction of insulin with its receptor is altered by the kinetic binding properties of the insulin analogue, and is apparently not the result of genetic abnormalities within the target cells themselves.

There is evidence for a similar dependence of signalling specificity on the timing properties of events downstream of hormone binding. In PC12 rat pheochromocytoma cells, epidermal growth factor and nerve growth factor appear to share

Abbreviations used: IRS, insulin receptor substrate; MAPK, mitogen-activated protein kinase.

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Scheme 1 Positive and negative signals in the insulin signal-transduction network

The diagram shows the existence of branches and feedback loops in the signalling network. For a detailed explanation, see recent reviews [4,5]. MAPK, mitogen-activated protein kinase; MEK, MAPK kinase; MKP, MAPK phosphatase; TNF α , tumour necrosis factor α ; IRS, insulin receptor substrate; PI3K, phosphatidylinositol 3-kinase; PTPase, protein tyrosine phosphatase.

many common components in the MAPK signalling pathway. However, epidermal growth factor primarily stimulates proliferation and triggers a transient activation of MAPK, whereas nerve growth factor primarily stimulates differentiation and triggers a sustained activation of MAPK [26–29]. That is, specificity of signalling appears to depend on the duration of activation of a component of a common pathway.

One interpretation of the dependence of signalling properties on dissociation rate and on the timing properties of signalling events in general is that there is a process downstream of the event that requires a certain minimal time to become activated [2,30] (e.g. nuclear translocation of MAPK in PC12 cells [29]). If two such processes are activated simultaneously, a ‘critical race’ [31] develops, in which the faster of the two processes wins at the expense of the slower. For example, binding of hormone might activate the receptor to promote the accumulation of some activator at a characteristic production rate, and signalling occurs only when the activator exceeds a threshold level. If the receptor remains activated through its association with hormone for a sufficiently long time, the threshold level is attained and the corresponding signalling pathway is taken. If, instead, the hormone dissociates and the receptor becomes deactivated before the critical process is triggered, there will be no signal at all, or an alternative pathway that was triggered at a lower threshold will be chosen. The rationale for treating biological interactions as switches with well-defined on and off switching times stems from the observation that the effect of a regulator typically changes from inefficient to efficient over a rather small range of concentration, thereby giving rapid switching not only with respect to concentration but also with respect to time as the regulator concentration varies (see, e.g. [32–34]).

If this type of decision-making occurs at multiple points along the activation pathway(s), the end result is a complex com-

bination of selections among alternatives based on their timing properties. Furthermore there could be feedback loops in the signalling pathway in which downstream events influence the triggering of earlier events in the pathway. Examples of such feedback loops in insulin or growth factor signalling pathways are the desensitization of insulin-activated p21^{ras} by MAPK kinase-induced dissociation of the Sos–Grb2 complex despite persistent receptor activation [35,36], or the negative regulation of MAPK by MAPK phosphatase, the mRNA of which is stimulated by MAPK (see [37]). Such feedback loops introduce further complexity into the decision-making apparatus, and lead to additional dependencies on the timing properties of the interactions.

It can be difficult to understand intuitively how the timing of signalling events (including that of receptor binding) may affect ‘decision-making’ at branching pathways, and a logical demonstration of this concept is warranted. We attempt here such a demonstration, with the goal of providing a simple method for the formal quantitative description of timing-dependent signalling networks. Complex metabolic biochemical networks have traditionally been analysed in terms of systems of coupled kinetic differential equations [38–40], but although such systems are easily simulated using modern computers, their behaviour rapidly becomes difficult to comprehend as the number of components increases. This has led to promising alternative approaches based on switching circuit theory [33,34,41–46], topological theories [47] or hybrid approaches using combined logical/kinetic methods to analyse specific biological problems [31,48,49]. Several of these studies have investigated the equivalence of the kinetic and logical methods, and have shown how the logical formalism can predict complex behaviour such as the appearance of multiple steady-states or oscillations.

We propose here a logical formalism for describing the

behaviour of receptor signalling systems, in particular those of the insulin receptor, underlining the role played by timing in determining that behaviour. Our formalism is based on the approach developed by Thomas and co-workers for genetic networks [33,34,46], which emphasizes the importance of asynchronous switching and feedback loops in interacting networks. In the work presented here, a logical network is constructed from the qualitative properties of the signalling interactions, taking the timing of successive events into account. Conditions for selecting alternative routes at each branching point in the pathway are derived, and the overall conditions for traversing pathways leading to alternative cellular behaviour are deduced. Using this approach, we present a simple logical network representing mitogenic signalling through the insulin receptor, in which times for activation and deactivation of individual components of the pathway are explicitly considered. We derive quantitative conditions for the selection of alternative signalling pathways based on their timing properties, and relate these conditions to the kinetic properties of the binding of hormone to its receptor.

DEFINITION OF THE LOGICAL SYSTEM

For simplicity, we consider only a three-step process: ligand binds to the receptor, the receptor becomes activated and the activated receptor transmits a biological signal if activated long enough. In the example of insulin signalling, we assume that it is the mitogenic signal that depends on the residence time of the hormone on the receptor. Metabolic signalling is assumed to take place either at a lower threshold signal or through another pathway entirely, and will not be explicitly included in the development presented here. In this basic formulation, we do not consider subthreshold binding events leading to no signal, nor will we consider forms of feedback within the signalling pathway.

Description of logical variables

We define the state of the system in terms of three Boolean variables which can take the values 0 or 1: b = receptor occupied by ligand (1) or not (0), a = receptor activated or not, and s = signal (i.e. mitogenic signal) transmitted or not. The state of this receptor system is then described by a three-digit binary vector, bas , specifying the values of the Boolean variables. The system we are considering can be represented by the interaction diagram (or 'graph') shown in Scheme 2, where the + signs indicate a positive effect on the target variable; that is, the bound state ($b = 1$) stimulates the activated state of the receptor, and the activated state ($a = 1$) stimulates the signalling. In this notation, inhibitory (negative) effects would be indicated by a minus (−) sign. The positive feedback of s on itself is present to ensure that once the signal is activated, it will continue through downstream processes independent of the subsequent fate of the antecedent variables b and a . That is, once $s = 1$, the signalling is fully committed.

Note that the interaction graph given in Scheme 2 explicitly shows only the forward positive reactions. For a complete representation of the signalling system, we need also to consider the reverse reactions, that is, the decay of a state in the absence of positive input. To do this, we assume in general that, if a state

variable is activated (i.e. its Boolean value is 1), it will become deactivated (Boolean value 0) after some characteristic time in the absence of positive input [34]. For example, the receptor activation state will switch off at some time after ligand dissociates (for example, as the result of the action of protein phosphatases on the receptor tyrosine kinase).

Dissociation of ligand

The dissociation of ligand, or more explicitly, the residence time of ligand on the receptor, can be represented in an identical manner. Once ligand is bound, it will tend to dissociate in a characteristic time, which is inversely related to its dissociation rate constant k_d . Under the ordinary assumptions of Michaelis–Menten binding, this time is independent of the continuing presence of free ligand, or of the free ligand concentration. That is, the receptor-bound state variable b also follows the general pattern that an unstimulated state decays in some characteristic time, and allows us to model what is really occurring, namely, that ligand becomes bound but can thereafter dissociate even in the continuing presence of free ligand. We do not consider in the basic formulation the possible influence of co-operativity in binding on the dissociation rate and residence time of the ligand [14]; rather we take the characteristic residence time as a fixed value.

Note that in a real biological system, the ligand residence time (as well as other time delays in the signalling pathway) will not be fixed, but rather will have a distribution of values. Therefore, within a population of receptors, there can be a distribution of behaviour which is related to the distribution of ligand residence times. The overall population behaviour can be quite complex because of the non-linear and discontinuous nature of the state transitions, but for any particular state transition dependent on a single transition time parameter, the average behaviour should in general follow the distribution of that time parameter; that is, the fraction of receptors making the transition should increase or decrease as the time distribution shifts left or right. In this work we assume that the state transitions can be approximately described in terms of their average behaviour, and therefore represent transition times as fixed time parameters, which in the case of the ligand residence time is inversely related to the dissociation rate constant.

Logical image state

As mentioned above, the state of a system is defined by the logical values (in simple cases, 0 or 1) of the variables b , a and s , in other words by the value of the state vector bas . For example, if the state vector is 100, it means that the receptor is bound ($b = 1$), that it is not activated ($a = 0$) and that the signalling is not committed ($s = 0$).

However, in order to describe the dynamics of the system, one has to consider as well the 'image' vector [42], which we denote as BAS . This vector represents the state toward which the system tends under the influence of the state interactions. For example, if the state vector is 100 as above and the image 010, it means that the receptor is bound ($b = 1$) but that it has a command ($B = 0$) to dissociate (either because of its natural tendency to dissociate, or perhaps because the external concentration of the ligand has dropped below a threshold value), that the receptor is not activated ($a = 0$) but that (in view of the fact that the ligand is still bound) there is a command ($A = 1$) to activate the receptor, that the signalling is not committed ($s = 0$) and that, since $S = 0$, there is so far no command to change this situation.

At first glance, one might suppose that the image of the present state is nothing else than its next state. This attitude (which is

Scheme 2 Interactions among the state variables

adopted in so-called ‘synchronous’ logical descriptions (see, for example [41,43]) would imply that all the commands are obeyed after exactly the same time delay. Without excluding the possibility of such a coincidence, we consider it marginal, and assume that, in general, time delays are different from each other. The above situation can be described as *100/010*. This state is thus subject to two commands, a switch of *b* from 1 to 0 and a switch of *a* from 0 to 1. Since there is no reason whatsoever for these two processes to take place at exactly the same time, we reason that the next state will be *000* or *110* depending on which command has been executed first; state *100* thus has two potential next states.

To summarize, the image of a state is the state toward which the system would tend if the commands persisted throughout. However, since the commands are executed one at a time, the system usually proceeds from a ‘present’ state to a next state in which only one of the commands implied by the image has been executed; in this new state, the commands are usually different from what they were, and in particular some commands may have disappeared (‘counter-order’). Thus the image of a state will generally not be its next state, and in many cases a state has a ‘choice’ between two or more next states. Which one is actually chosen will depend on the values of the time delays. All this will become much more concrete once we have shown how the present state and its image are formally related by the logical equations.

Logical transition rules

From the interaction graph (Scheme 2), and the assumption that unstimulated variables tend to decay, we write down a set of logical rules that define the logical transitions, and hence the image state for each possible system state. In the formulation presented here, we model only the signalling behaviour of a receptor for a single ligand-binding event; we do not consider the effects of repeated binding. Therefore the image state always contains $B = 0$, which means that *b* tends to 0 if it has the value 1 (i.e. ligand tends to dissociate), and *b* remains 0 if it has the value 0 (no ligand rebinding considered in this model). Under these assumptions, the rules are as follows for Scheme 2:

$$\begin{aligned} B &= 0 \\ A &= b \\ S &= a + s \end{aligned} \quad (1)$$

In these and later equations we use the notation that a logical inclusive *OR* relationship is expressed as $X = y + z$, and a logical *AND* relationship is expressed as $X = yz$. The first relationship in eqns (1) states that *b* always tends toward 0, as described above. The second rule states that *A* is true ($A = 1$) if *b* is true ($b = 1$); otherwise *A* is false ($A = 0$). In other words, the receptor activation state tends toward 1 if the ligand is bound; otherwise it tends toward 0. The last rule states that *S* is true if either or both of the conditions *a* or *s* is true. This means that mitogenic signalling tends toward 1 either if the receptor is activated ($a = 1$) or if mitogenic signalling has already been initiated (i.e. signalling, once started, is fully committed).

Transition truth table and state transition diagram

To develop the logical formalism, we construct a table, shown in (2) below, giving the complete list of possible states and their associated image states, computed using eqns (1).

<i>b</i>	<i>a</i>	<i>s</i>	<i>B</i>	<i>A</i>	<i>S</i>
(0	0	0)	0	0	0
(0	0	1)	0	0	1
0	$\bar{1}$	$\bar{0}$	0	0	1
0	$\bar{1}$	1	0	0	1
$\bar{1}$	$\bar{0}$	0	0	1	0
$\bar{1}$	$\bar{0}$	1	0	1	1
$\bar{1}$	1	$\bar{0}$	0	1	1
$\bar{1}$	1	1	0	1	1

(2)

A variable *b*, *a* or *s* will tend to change when its value differs from that of its image, and the direction of this change is indicated in the table by a + or – sign placed over the variable. For example, if *b* is 0 (no ligand bound), this will influence the activation state *a* toward 0 (deactivation). This notation expresses the same information contained in the image state, but is a convenient way of directly indicating the pending state transitions in the system [34]. Parentheses around a state indicate that the state is stable under the given input conditions.

PROPERTIES OF THE LOGICAL SYSTEM

Branching in the logical pathways

If the image of a system state contains more than one binary digit different from that of the system state itself, each of the corresponding Boolean variables will individually tend to switch as the result of the system interactions. Because we assume these switching reactions proceed independently, the variables will in general not switch at precisely the same time, and the next state achieved by the system will not be the image state itself, but a state with only one Boolean variable different from the original system state. In particular, the switched variable in the new state will be the one with the shortest switching time, i.e. that variable will have won the ‘critical race’ [31]. At this new state, the system interactions dictate a new set of interactions and a new image state, and the process repeats itself.

If we consider the value of the characteristic switching times as unknown, any of the Boolean state variables might be the first to switch, if its switching time happens to be the shortest. This situation thus represents a logical branch in the signalling pathway, and which arm of the branch is taken will depend on the timing properties of the transitions. We assume that each switch of a Boolean variable, either *on* or *off*, requires a fixed characteristic time, and if the signal driving the switch is present for less than this time, the switch does not occur. (For a single signalling event, the time delays will have well-defined values, but in a population of receptors, or over a period of time, these values will in general be distributed, leading to a distribution of behaviours among the receptors in real systems.)

For example, if the receptor becomes bound, and remains so, it will become activated after a time t_a ; if the ligand dissociates before this critical time, no activation occurs. Similarly, we define t_s as the characteristic time for initiation of signalling after the receptor becomes, and remains, activated. The characteristic times for decay of the state variables *b* and *a* in the absence of positive input are designated by t_b and t_a respectively. Note that the time t_b will not appear in this model since we do not consider

Scheme 3 Transition diagram showing all possible state transitions

Under each state is shown the total time to reach that state relative to time 0, in terms of the characteristic state variable transition times. States are identified (a)–(j) for reference in the text. Circled states represent the steady states of the system.

ligand rebinding, and the time t_s will not appear since we assume signalling is committed once initiated.

Logical transition diagram

To derive the conditions for signalling for a single ligand-binding event, we start from the state (100), i.e. where receptor has just become bound by ligand and is neither activated nor signalling. Starting from this state, a complete transition diagram can be drawn to show all logically possible state transitions as shown in Scheme 3.

Branches occur wherever there are two state variables being driven to switch. For this system, all branches have only two arms; in other systems, however, there could be multiple arms at a branch point. In this transition diagram, there are two possible biological end states, one with no signalling (state 000, reached either through Path 1 or Path 2) and one with signalling (state 001, reached through Paths 3 or 4). Therefore the conditions for the absence of mitogenic signalling, or its occurrence, will be expressed as conditions for traversing either Path 1 or Path 2 versus traversing Path 3 or Path 4.

Calculation of transition times

By our assumptions, at each branch, the arm requiring the least time will be traversed, and the condition for choosing one arm instead of another can be expressed as an inequality between the times for traversing the respective arms of the branch. To derive these conditions we take the starting time at state 100 (i.e. ligand has just bound to an inactive receptor) as $t = 0$, and calculate the total time for reaching each succeeding state in terms of the characteristic transition times of the Boolean state variables. The conditions for selection of each branch arm are then expressed in terms of inequalities among these total times. To correctly calculate these times, we note that each segment of the transition diagram represents a switch of exactly one Boolean variable, which occurs in a fixed characteristic time, say t_x . Therefore the target state is reached at a time exactly t_x later than that of the state at which the signal for the switch was initiated. Note that this signal may not have been initiated at the immediately preceding state, but may have been pending for one or more state variable transitions, i.e. one or more segments of the transition diagram. Following this procedure, a time for reaching every state in the transition diagram can be written in terms of the characteristic state variable switching times, and the inequality conditions for traversing any particular arm of a branch are thus directly derived.

Branching decisions

Since any complete pathway leading to a biological end point involves several successive branching decisions, the complete condition for traversal of a pathway becomes the logical *AND* of the conditions for selection of each branch along the path.

As an example, we calculate the conditions for traversal of the pathway marked Path 2 in Scheme 3. First, the branch to the state marked (c) must be taken. The condition for this [i.e. switching to state (c) instead of (b)] is:

$$t_a < t_b \quad (3)$$

Then the branch to state (d) must be taken, which has the condition:

$$t_b < t_a + t_s \quad (4)$$

and finally the branch to state (f) must be taken, with the condition:

$$t_b + t_a < t_a + t_s \quad (5)$$

The full condition for traversal of Path 2 is the logical *AND* of these conditions, namely:

$$(t_a < t_b)(t_b < t_a + t_s)(t_b + t_a < t_a + t_s) \quad (6)$$

Using this procedure, we can derive the conditions for all four of the possible pathways.

To simplify the notation, we define the logical variables m , n and q as follows:

$$\begin{aligned} m &\equiv (t_b < t_a) \\ n &\equiv (t_b < t_a + t_s) \end{aligned} \quad (7)$$

$$q \equiv (t_b + t_a < t_a + t_s)$$

For example, the variable m will equal 1 (*true*) if t_b is less than t_a , and will equal 0 (*false*) otherwise. Using this notation, the conditions for each of the pathways become:

Path 1: m

Path 2: mnq (8)

Path 3: $m\bar{n}\bar{q}$

Path 4: $\bar{m}\bar{n}$

Path 1 and Path 2 are alternative routes to one biological end point (no proliferative signalling), and Path 3 and Path 4 are routes to another biological end point (occurrence of proliferative signalling). Therefore the conditions for the alternative biological end points are:

$$\text{No proliferative signalling: (Path 1) + (Path 2) = } m + mnq \quad (9a)$$

$$\text{Proliferative signalling: (Path 3) + (Path 4) = } m\bar{n}\bar{q} + \bar{m}\bar{n} \quad (9b)$$

where $+$ means logical *OR* as usual.

These expressions can be considerably simplified, using the following procedure (see [33,50]).

1. We first note that, since time delays are all positive numbers, t_a is always less than $t_a + t_s$, which means that, referring to the definitions of m and n in relationships (7), if m is true, then n is also automatically true; that is, m implies n . In standard notation:

$$m \Rightarrow n \quad (10)$$

Note that this relationship is equivalent to $\bar{n} \Rightarrow \bar{m}$. Using similar reasoning applied to the definitions of n and q in relationships (7), we see that

$$q \Rightarrow n \quad (11)$$

From these relationships, we deduce that the conditions $m\bar{n}$ or $q\bar{n}$ can never exist in our system.

2. Using the logical expression (9a), we now construct a logical table giving the conditions leading to the absence of proliferative signalling, in terms of the values of the three logical variables m , n and q , as follows:

	0,0	0,1	1,1	1,0	m, n
0	0	0	1	–	(12)
1	–	1	1	–	
q					

The values of m and n are indicated along the top, and the values of q are indicated down the left. The entries are the values of the expression (9a), so that a 1 means that no proliferative signalling is predicted. Impossible combinations of the logical variables, due to the relationships (10) and (11), are marked with dashes.

3. The logical content of the table can now be determined by inspection. The absence of proliferative signalling is predicted for all conditions where a 1 is found in the logical table (12), and it can be seen from the table that this occurs wherever q is 1 or m is 1. In logical notation therefore the conditions for no proliferative signalling can be simply expressed as:

No proliferative signalling: $m + q$

 (13)

4. Similarly, using the expression (9b), we construct a logical table giving the conditions for proliferative signalling, as follows:

	0,0	0,1	1,1	1,0	m, n
0	1	1	0	–	(14)
1	–	0	0	–	
q					

5. Proliferative signalling is predicted for all conditions where a 1 appears in the logical table (14), and it can be seen that this occurs where both m and q are 0. In logical notation, this can be summarized as:

Proliferative signalling: $\bar{m}\bar{q}$

 (15)

Note that because the two possibilities, proliferative signalling or not, are the complement of each other, the two logical tables (12) and (14) are also complementary; that is, wherever a 1 appears in one table, a 0 appears in the corresponding place in the other table. Note also that the simplified logical conditions (13) and (15) could have been derived directly from the expressions (9a) and (9b) using standard theorems of symbolic logic (see, for example, [33]), plus the constraints (10) and (11).

In terms of the characteristic transition times, the condition for proliferative signalling given by (15) is:

$$(t_a < t_b)(t_a + t_s < t_b + t_a)$$

Expressed as a condition on the residence time, t_b , of ligand on the receptor, this becomes:

$$(t_b > t_a)(t_b > t_a + t_s - t_a)$$

which is equivalent to:

$$t_b > \max(t_a, t_a + t_s - t_a)$$

This says that for mitogenic signalling, the residence time of ligand on the receptor must be greater than the maximum of two quantities, the first of which is the activation time, and the second of which is the sum of activation time plus proliferation commitment time minus activation decay time. In a simple case where the time for proliferation commitment, t_s , is less than the decay time of activation, t_a , so that $t_a > t_a + t_s - t_a$, the condition for proliferative signalling reduces to:

$$t_b > t_a$$

which is just the condition that a receptor is occupied by ligand long enough to become activated. That is, under these conditions, any receptor that becomes activated will send a proliferative signal.

DISCUSSION

We have presented a simple form of logical switching theory which incorporates the key point of the insulin receptor metabolic versus mitogenic signalling that we are trying to model; namely, that long residence time of the ligand on the receptor appears to correlate with enhanced mitogenic signalling. The theory then gives inequality relationships among the temporal parameters of the interacting components giving conditions for mitogenic signalling, or its absence, which we take to imply metabolic signalling. In our formalism, time delays for activation and deactivation have been explicitly considered, so that experimental measurement of the relevant time intervals can provide data to insert into the theory. Such data are not generally available yet for hormone receptor signalling systems, although examples of the kind of kinetic data required can be found for systems such as *Escherichia coli*, where measured rates of gene transcription can be used to estimate time delays in the expression of interacting genes in the bacteriophage λ lysis/lysogeny decision network (see e.g. [31]). Given measurements of critical times for switching of intracellular signalling events under different experimental conditions, predictions can be made of the selection among alternative pathways in the signalling cascade under those conditions.

This kind of analysis can also be carried out in reverse. If the basic structure of the signalling pathway is correctly formulated in the theory, then observation of specific types of switching or specificity can lead to predictions of the expected activation or deactivation times for reaction components, which can help in resolving questions of detailed interactions in the pathway. In the example presented here, one state variable is receptor 'activation', but the exact nature of this activation is unspecified. Given that a particular signalling selectivity behaviour is observed, limits on the time scales of the positive and negative switching of the activation step are derived. This can then be used to help narrow down the choice of possible candidate reactions which might represent the receptor activation, based on the range of characteristic times known to be typical for those reactions.

In our description, we have only considered the signalling resulting from a single ligand-binding event; that is, we start with a bound receptor and allow the ligand to dissociate but not to rebound to the receptor. In a real biological system, ligand is typically present at some concentration, and therefore will rebound to the receptor at a rate that is directly dependent on that concentration. If the time scale of the downstream cascade of events is short compared with the reassociation time of the ligand (e.g. at low ligand concentration or low kinetic association rate), the selectivity of signalling will be established before ligand has a chance to rebound, and the results presented here will be valid subject to the correctness of the other assumptions. On the other

hand, if ligand rebinding is rapid, multiple binding of ligand might occur during the course of the signalling-decision process. This possibility complicates the analysis and will not be further considered here. However, we have shown [51] that such a situation is easily handled in our formalism by simply introducing a negative feedback of the binding step on itself, which causes the unbound receptor ($b = 0$, see above) to become bound ($b = 1$) at a characteristic rate dependent on the free ligand concentration and kinetic association rate constant, and causes the bound receptor to become unbound at a rate dependent on the kinetic dissociation rate constant. Even with this rather simple extension of the model, it can be difficult to visualize the properties of the system intuitively, but, with the techniques shown here, a full analysis can be performed relatively straightforwardly.

The logical system developed here shows how the timing of biological events in the signalling pathway can be taken into account in determining the specificity of receptor signalling. In our example, that of proliferative signalling through the insulin receptor, we have expressed the conditions for transmission of the proliferative signal in terms of the delay times required for initiation or decay of components of the signalling pathway. Although the simplified example we have chosen includes only three events, namely ligand binding, receptor activation and triggering of the proliferative signal, and we have focused in particular on ligand residence time on the receptor, additional more specific events could have been included in the formalism. The advantage of this approach can clearly be seen for analysis of biological systems with more complex interaction networks. The logical structure can be built up systematically, and the possible signalling outcomes can be computed unambiguously from that structure. In fact, such a procedure can be incorporated into a computer program, allowing the analysis of more complex interacting structures ([51]; R. Shymko, unpublished work). There are a number of possible types of interaction, such as having multiple inputs for events in the signalling chain, or feedback loops from later to earlier events, which make it difficult to fully understand the properties of a signalling cascade without a formal method of analysis. The approach described here should be of help in developing such an understanding.

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