Bistable switching and excitable behaviour in the activation of Src at mitosis

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ABSTRACT

Motivation: The protein tyrosine kinase Src is involved in a multitude of biochemical pathways and cellular functions. A complex network of interactions with other kinases and phosphatases obscures its precise mode of operation.

Results: We have constructed a semi-quantitative computational dynamic systems model of the activation of Src at mitosis based on protein interactions described in the literature. Through numerical simulation and bifurcation analysis we show that Src regulation involves a bistable switch, a pattern increasingly recognised as essential to biochemical signalling. The switch is operated by the tyrosine kinase CSK, which itself is involved in a negative feedback loop with Src. Negative feedback generates an excitable system, which produces transient activation of Src. One of the system parameters, which is linked to the cyclin dependent kinase cdc2, controls excitability via a second bistable switch. This topology allows for differentiated responses to a multitude of signals. The model offers explanations for the existence of the positive and negative feedback loops involving protein tyrosine phosphatase alpha (PTPα) and translocation of CSK and predicts a specific relationship between Src phosphorylation and activity.

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INTRODUCTION

One striking feature of the protein tyrosine kinase pp60v-c-Src is the number of biochemical signalling pathways it is involved in. Cellular functions such as cytoskeletal organisation, cell cycle control, growth factor signalling, cell adhesion, migration and differentiation depend on it (Thomas and Brugge, 1997; Bjorge et al., 2000; Brown and Cooper, 1996). The numerous phosphorylation targets that have been identified characterise Src as a hub in cellular signalling networks (Abram and Courtneidge, 2000).

It was originally discovered as a viral oncogene (v-Src) (Rous, 1911) and in fact it is frequently deregulated in various human cancers (Irby et al., 1999; Thomas and Brugge, 1997). Its normal, physiological form, c-Src, occurs in all higher vertebrates. It also constitutes a family of tyrosine kinases consisting of nine members. Src homology domains (SH domains) appear as interaction domains in many other proteins. For example, SH2 is an important phosphotyrosine (pTyr) binding motif. Crystal structures of Src family proteins have provided many clues as to its regulation (Xu et al., 1997).

However, despite its obvious importance to the correct functioning of cellular processes, a tangible role for Src in cell physiology has not yet emerged.

Thomas and Brugge identified six major difficulties that impede the functional characterisation of individual Src family kinases (Thomas and Brugge, 1997). Among these are: redundancy among Src family members, the complexity of their downstream pathways, interference due to a high degree of homology among interacting proteins and a few others that make the in vivo system inaccessible.

Our computer-aided approach aims to tackle this complexity through mathematical modelling and numerical analysis. We present here a dynamic systems model aiming to characterise the activation of Src at mitosis by simulating known and hypothesised interactions.

A dynamic systems model describes the temporal evolution of a system state using only a fixed set of rules. In a protein-protein interaction model the system state is defined by the concentrations of each molecular entity at a given time t. The dynamic rules of the model allow computation of a subsequent state at a later time t + Δt.

The objective of our approach is two-fold: First, complex biological systems require a reliable way of testing whether the hypothesised interactions account for the observed behaviour in the real system. The aim is to find reasons for the vast complexity of interactions that Src undergoes in the characteristics of its temporal behaviour. Second, dynamic models have predictive capabilities. A variety of experimental set-ups, such as overexpression and deletion or the effects of amino acid substitution, can be simulated with our model. Emergent properties such as bistability, robustness or sensitivity to system parameters, such as kinase activities or physiological conditions, can be verified in wet-lab experiments and thus provide new insights about the characteristics of the enzymes involved.

Current research is beginning to relate known protein-protein interactions to architectural concepts and principles (Tyson et al., 2003; Milo et al., 2002). One important concept is bistability, which is now recognised as an essential feature of cellular signalling networks (Bhalla and Iyengar, 1999). A bistable system can alternate between two discrete stable steady-states in response to a signal. Bistable biochemical switches can, for example, amplify and modulate an extra-cellular signal to yield a decisive all-or-nothing response from the intracellular logic. Enzymatic systems involved in cell cycle regulation appear to make heavy use of bistable systems in order to co-ordinate progression through each stage of the cycle (Fuß et al., 2005; Ingolia and Murray, 2004).

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The Src system

As noted above, Src interacts with a huge number of different proteins. From these we have taken an exemplar set that is thought to be essential to the activation of Src at mitosis.

We know that Src activity is controlled in a complex manner through several phosphorylation sites: When phosphorylated on Tyr529, Src assumes a compact, inactive conformation due to an intramolecular pTyr-SH2 interaction (Xu et al., 1997). It is normally inactive in this conformation (with exceptions as we shall see later). The N-terminal sites Thr34, Thr46 and Ser72 are thought to be phosphorylated by the cyclin-dependent kinase cdc2 (Shenoy et al., 1989). Their phosphorylation leads to weakening of the intramolecular pTyr-SH2 interaction and exposes pTyr529 to phosphatases (Stover et al., 1994; Shenoy et al., 1992). Phosphorylation of the other major tyrosine phosphorylation site, Tyr418 within the activation loop, is known to significantly increase kinase activity (Kniecck and Shalloway, 1987; Xu et al., 1999). This site is phosphorylated in an autocatalytic reaction.

Two other proteins are associated with phosphorylation and dephosphorylation of Tyr529: PTPα and CSK (see Fig. 1). PTPα (Protein Tyrosine Phosphatase alpha) is the ubiquitously expressed receptor protein tyrosine phosphatase (RPTP) that is known to dephosphorylate Src pTyr529 and thus to positively affect Src activity. Furthermore it has been suggested that PTPα is a target of Src itself: PTPα features at least one phosphorylation site at Tyr789 that is essential for its Src directed activity. It has been hypothesised that PTPα therefore participates in a positive feedback loop with Src (den Hertog et al., 1994; Zheng and Shalloway, 2001). The activity of PTPα is further regulated by an inhibitory protein, Grb2. Grb2 binding to PTPα down-regulates its activity (den Hertog et al., 1994; Zheng and Shalloway, 2001). However, serine hyperphosphorylation of PTPα can prevent their interaction. Zheng and Shalloway suggested that protein kinase C (PKC) could be responsible for this modification (den Hertog et al., 1994; Zheng and Shalloway, 2001).

CSK (c-Src tyrosine kinase or C-terminal Src kinase) is a negative regulator of Src activity and is homologous to Src. However, its activity is regulated in a different fashion. In the inactive state, CSK resides in the cytoplasm. Cbp (CSK-binding protein, also known as PAG), a membrane-located binding protein and phosphorylation target of Src, associates with CSK through a pTyr-SH2 interaction. This interaction enables its kinase activity and recruits it to the membrane, where it can target the inhibitory Src phosphorylation site Tyr529. Cbp and CSK therefore seem to constitute a negative feedback loop with Src (Howell and Cooper, 1994; Kawabuchi et al., 2000; Brdička et al., 2000).

METHODS

Information retrieval

Our model is the result of a hypothesis-driven (as opposed to data-driven) approach. The information about protein interactions was mainly retrieved from published articles referenced throughout this paper.

Default parameter values were manually adjusted to fit observations from literature and to correspond to physiologically plausible assumptions. Where available, quantitative findings from literature have been accommodated. However, our model focuses on the discovery of general, qualitative behavioural features rather than on making quantitative predictions. The model variables therefore represent dimensionless concentrations.

Computational modelling

The software tool Narrator, an implementation of the Codependence Model formalism (Mandel et al., 2006), was used to define the Src system model in Fig. 2.

Codependence Models are a graphical formalism intended for describing dynamic models of complex biological systems. The main strength of the formalism is the uniform description of transport or (for example chemical) transformation processes and informational interactions, such as stimulation and inhibition (Mandel et al., 2006). The formalism also defines a dynamic interpretation and therefore allows direct translation to a system of ordinary differential equations (ODE). It is more readable and less prone to errors, since features like conservation of mass are implicitly derived from the graph structure. The equations that need to be specified (see Table 1) are much simpler than in a typical biochemical ODE model. Unlike many formalisms, its model elements are abstract entities rather than representations of concrete biochemical classes such as enzymes, proteins, transcription factors etc. Its one-to-one correspondence to ODEs enables the use of a full range of mathematical analysis techniques. The main elements of the Codependence Model formalism are described in the caption of Fig. 2.

Kinetic equations

Our model employs simple, mass-action based kinetics to represent enzymatic reactions. The resulting equations are simpler than for example Michaelis-Menten kinetics and contain fewer parameters. This simplification is valid under the assumption that the Michaelis constant KM for each enzyme is large compared to its physiological concentration.

All kinetic equations (velocities) therefore take the form

\[ v = \sum v_i \cdot k_i \cdot d_{\text{enzyme}} \cdot \text{substrate} \]

where \( v_i \) is a stoichiometric parameter to distinguish forward and reverse reaction, \( k_i \) the kinetic rate constant and \( d_{\text{enzyme}} \) the activity of the relevant enzyme. Enzymatic activities of Src and PTPα also include a background element \( d_{\text{enzyme}}^0 \).

Numerical simulation

For subsequent mathematical analyses, the model was converted to an ODE file for use with XPPAut (Version 5.91) (Ermentrout, 2005). The latter is a numerical simulation program for ODEs and other types of equation systems. It includes an interface to AUTO (Doedel, 1981), a software package for numerical bifurcation analysis, which was used to generate Figures 3 to 7.

For numerical solution of differential equations a fourth order Runge-Kutta algorithm with adaptive step size was used, with a maximum step size of 0.1. AUTO was operated with an error tolerance of 10^{-7} (EPSL, EPSU and EPSS) and a step size not greater than 0.01.
RESULTS

Model definition

We have constructed a dynamic model that represents interactions between Src, PTPα and CSK. These proteins were selected because of their known and described importance for tyrosine phosphorylation in the cell cycle, specifically at mitosis (Mustelin and Hunter, 2002). Zheng and Shalloway developed a basic temporal model for PTPα activation (Zheng and Shalloway, 2001, Fig. 10). Our approach integrates this model and the Cbp/CSK system (see for example Kawabuchi et al. (2000); Okada et al. (1991)) in a computational dynamic systems model.

A diagrammatic representation of the model, for which we have used a graphical formalism called Codependence Models (Mandel et al. (2006), see Methods), is shown in Fig. 2. The model consists of ten species, which hold the concentrations of each molecular entity. The model works with fractional units rather than physiological concentrations, since there is little quantitative data available. These entities are connected through seven reactions (process clouds in Fig. 2). Their velocities, \( n_1 \) to \( n_7 \), are defined using only basic mass-action kinetics (Table 1). Kinetic parameters are indicated as weights on the respective links (thin lines, known as conditional links).

Src occupies the central role in our model. Two tyrosine phosphorylation sites are of particular importance to the dynamics of Src activation, namely Tyr529 and Tyr418. These two sites allow four possible combinations of phosphorylation states, which are depicted on the left-hand side of Fig. 2. Other phosphorylation sites are not explicitly represented in the model, but the effect of N-terminal serine and threonine phosphorylation can be simulated through model parameters as we will see. The four states are abbreviated \( s_{ri} \) (inactive), \( s_{ro} \) (opened), \( s_{ra} \) (activated) and \( s_{rc} \) (closed) for convenience. The corresponding states of each phosphorylation site can be seen in Fig. 2.

The model allows all conformations except \( s_{ri} \) to contribute to kinase activity, and each to a different degree, reflected by their specific molar activities \( r_{sro}, r_{sra} \) and \( r_{scc} \). The node ‘Src activity’ combines these into a summative activity exerted onto Src phosphorylation targets, which includes Src itself (autophosphorylation, interaction E). Tyrosine kinase background activity is included as an additional model parameter.

Phosphorylation status of Tyr529 is controlled by CSK (interactions A and B in Fig. 2) and PTPα (interactions C and D). We assumed that the activity of these enzymes is independent of Tyr418 phosphorylation status, hence they control both \( v_1 \) and \( v_3 \).

While the \( s_{rc} \) state (phosphorylated on both Tyr529 and Tyr418) has been observed \textit{in vitro} (Boerner et al., 1996), its exact role \textit{in vivo} remains controversial. The kinase domain of \( s_{rc} \) appears to retain its activity. Once Src is activated, CSK is not able to deactivate it until pTyr418 is dephosphorylated (Sun et al., 1998). Xu et al. state that the \( s_{rc} \) state has not been observed \textit{in vivo} (Xu et al., 1999). It is unclear why CSK should not target \( s_{ra} \) in living cells. If \( s_{rc} \) cannot be observed, this could be due to a short lifetime of this state. This is reflected in the model by a high default value of \( k_4 = 10 \), which leads to rapid hydrolysis of pTyr418 in \( s_{rc} \). We do not, however, propose or favour any particular mechanism of

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**Fig. 2.** Computational model. Blue rectangles represent molecular species, turquoise clouds denote reactions between them. Blue arrows represent material flow (positive direction is indicated by filled arrowhead). Thin red lines, so-called conditional links, represent informational flow, e.g. enzymatic activity. The small red circle indicates information source, e.g. the enzyme catalysing the reaction. Each condition has an associated weight that controls the intensity of the interaction (ellipses on conditional links). If unspecified, the weight is 1. Large turquoise circles represent constants or computed logical entities.
Table 1. Mathematical definition of model components. $v_1$ to $v_7$ refer to processes in Fig. 2. Combined with the model topology these equations can be converted to a set of differential equations for numerical analysis.

**Processes:**

$v_1 = k_2 \cdot aPTPa \cdot srci - k_1 \cdot cbp-P-csk \cdot srco$
$v_2 = k_3 \cdot aScc \cdot srco - p_1 \cdot srca$
$v_3 = k_1 \cdot cbp-P-csk \cdot srca - k_2 \cdot aPTPa \cdot srcc$
$v_4 = k_4 \cdot p_1 \cdot srcc$
$v_5 = (aScc + p_3) \cdot PTP\alpha - p_2 \cdot PTP\alpha-pTyr789$
$v_6 = k_{cbp} \cdot aScc \cdot cbp$
$v_7 = k_{csk.on} \cdot cbp-P \cdot csk - k_{csk.off} \cdot cbp-P-csk$

**Activities:**

$aScc = \rho_{src} \cdot srco + \rho_{src} \cdot srca + \rho_{scc} \cdot srcc + a_{src}^0$
$aPTPa = pIpy + a_{PTPa}^0$

**Default parameters:**

$\rho_{src} = 0, \rho_{src} = 1, \rho_{src} = 1$
$k_1 = 1.0, k_2 = 0.8, k_3 = 1.0, k_4 = 10$
$k_{cbp} = 1.0, k_{PTPa} = 1.0, K_{ser} = 1.0$
$k_{csk.on} = 0.1, k_{csk.off} = 0.01$
$a_{src}^0 = 0.0001, a_{PTPa}^0 = 0$
$p_1 = 0.05, p_2 = 0.15, p_3 = 0.035$

**Default initial conditions:**

$srci_{0} = 1, cbp_{P_{off}} = 0, csk_{0} = 1, PTP\alpha_{0} = 0$

all other species are 0 at $t = 0$

deactivation. Ubiquitination of active Src, causing protein degrada-
tion, has been suggested as an active such mechanism (Hakak and
Martin, 1999).

The upper right part of Fig. 2 is concerned with PTP\alpha. Src-
directed activity of PTP\alpha again depends on its phosphorylation
status: Phosphorylation of Tyr789 seems to increase specific activ-
ity towards Src; and phosphorylation on serine residues decreases its
inhibitory binding to Grb2 (Zheng et al., 2000). The hypothesised
phosphorylation feedback loop between Src and PTP\alpha is rep-
resented by interactions G, C and D in the model. We assumed
that the two modifications, Tyr789 and serine phosphorylation,
are independent of each other. The model therefore shows PTP\alpha
activities. These findings suggest that Src may be switched on and off through a

**Bistability in the activation of Src**

To study the effect of CSK on the system let us first consider
a smaller subsystem of this model, where CSK concentration is kept constant (open-loop model). This can be realised by cutting
interaction F or setting $k_{cbp} = 0$. CSK activity (represented by
$Cbp-P-CSK$) now becomes a model parameter and can be varied
independently.

Fig. 3 shows two phase-plane diagrams that demonstrate the
response of this subsystem to variation in CSK activity. The
lines indicate stable-state solutions (fixed points), where the system
is at rest. Heavy lines represent stable solutions and dashed lines
represent unstable solutions. When perturbed by a small amount,
the system will return to a stable fixed point on either branch.

The two saddle-node bifurcation points SN1 and SN2 enclose
a bistable region. For any CSK concentration within this region,
the system has two stable solutions and one unstable solution.
The system therefore exhibits hysteresis, which is characteristic
of bistable systems: When travelling leftwards along the lower
stable branch by decreasing CSK activity, Src remains inactive
until SN2 is reached. If we decrease CSK any further, the lower
stable fixed point disappears and the system moves towards the
upper stable branch, corresponding to high Src activity. If CSK
increases again, the system will proceed along the upper stable
branch until SN1 is reached and another transition occurs, bringing
the system back to the lower branch.

The two stable branches clearly separate high from low Src activ-
ity and this effect is even more pronounced for PTP\alpha activity. These
findings suggest that Src may be switched on and off through a

**Role of the negative feedback loop involving CSK**

We will now return to the full model and reactivate the negative feedback loop involving CSK translocation. The system is still
attracted to the stable manifolds of Fig. 3. High Src activity, how-
ever, causes activation of CSK, which leads the system back to its
original state.

This phenomenon is called excitabile behaviour and is well-
known in other disciplines of biological modelling. Examples are
systems which involve spatiotemporal pattern formation or signal
propagation, such as in the famous neural membrane potential model by Hodgkin and Huxley (1952).

The phase-plane diagram in Fig. 4A shows trajectories of the simulated system response to a drop inSrc-directed CSK activity. For default parameters, excitation occurs below a threshold of CSK ≈ 0.5. After Src activation, CSK activity is restored through phosphorylation of Cbp by Src and translocation of CSK near the membrane. The trajectory leads back along the upper stable manifold to the original state on the lower branch.

This represents what we might observe during normal progression through the cell cycle: transient activation of Src at mitosis, followed by low Src activity after cytokinesis. However, our simulations show that this system is capable of more differentiated responses that could account for cell cycle checkpoint responses, in which Src activity is sustained at high level for longer periods.

We obtain a qualitatively different response if we restrict the amount of available CSK or increase the dissociation constant $K_d$ for Cbp and CSK (Fig. 4B): As the system moves along the upper stable branch towards SN1, Src activity leads to complete conversion of Cbp into Cbp-P. Free Cbp is depleted before the system reaches the bifurcation point. It therefore comes to rest on the upper stable branch.

As we will demonstrate, several system parameters will allow us to achieve this kind of dynamic behaviour. This mechanism of obtaining sustained Src activity can be exploited by a regulatory system to produce diverse responses to environmental conditions.

**Influence of physiological parameters**

Let us examine the influence of the parameter $k_2$. It determines the rate at which Src is dephosphorylated on pTyr529 through the effect of PTPα, represented by $v_1$ and $v_5$ in Fig. 2 and Table 1. This parameter reflects the status of several Src phosphorylation sites located near the N-terminus. The cyclin-dependent kinase cdk2 (also known as cdk1) is associated with phosphorylation of these serine and threonine residues. When phosphorylated, the cleft between the C-terminal tail and the SH2 domain widens and Tyr529 becomes more accessible to dephosphorylation. $k_2$ is therefore a function of cdk2 activity and thus an important connection to cell cycle.

Fig. 4A demonstrates the influence of $k_2$ on bistability of the system. As $k_2$ increases the bistable range expands and is shifted to higher CSK activities. Low $k_2$ values (corresponding to low cdk2 activity and therefore weak interaction between PTPα and Src) result in a strong inhibitory effect of CSK.

For a high $k_2$ the position of SN1 exceeds the amount of total available CSK in the cell (dotted line) and Src activity is no longer sufficient to bring the system back to the lower stable branch. Any further increase in $k_2$ amplifies this effect. We can display this relationship between $k_2$ and Src activity at equilibrium in another bifurcation diagram (Fig. 5B), from which two new bifurcation points, SN3 and SN4 emerge.

To the left of SN3 we observe excitable behaviour due to strong negative feedback. Within the bistable region, CSK will initially keep Src activity low, but cannot restore the low Src state after excitation. Finally, SN4 represents the point, above which CSK by itself is not even capable of enforcing the low Src state and Src becomes constitutively active. A CSK deficient cell will generally display this phenotype (Imamoto and Soriano, 1993; Nada et al., 1993).

These results show that the system exhibits bistability on at least two different levels: activation of Src by CSK and control of excitability. Many other bistable signalling systems have been characterised to yield an all-or-nothing response similar to what can be seen in Fig. 3 (Bhalla and Iyengar, 1999; Laurent and Kellershohn, 1999). The system described here is capable of three qualitatively distinct types of behaviour: stable high, excitable and bistable.

We see a potential role for this new, bistable region in the G2/M cell cycle checkpoint. Src normally displays only transient activity during these cell cycle phases, but the regulatory system needs to guarantee that downstream events of Src are completed before proceeding to the next stage. For example, if delays occur due to unfavourable environmental conditions, the action of cdk2 will keep the system to the right of SN3. This means that Src activity will continue until cdk2 activity falls below this point. The low Src state, however, is robust to cdk2 variation: Src activation is only triggered...
by a drop in CSK activity: CSK switches the system on, while cdc2 controls when it is switched off.

**CSK translocation and robustness**

As we have seen, the fact that the amount of available CSK is limited creates a second set of bifurcation points that define another bistable region. Experimental evidence suggests that the Src system is in fact sensitive to this amount (Imamoto and Soriano, 1993; Nada et al., 1993). Surprisingly, in our model we find instead a remarkable degree of robustness with respect to CSK.

Fig. 5 demonstrates the influence of total CSK on the bifurcation points SN3 and SN4 (see Fig. 3). The two straight lines containing the bistable region correspond to the positions of SN1 and SN2 from Fig. 3. The dashed line represents the amount of available CSK. At a critical value of \( k_2 \approx 0.67 \) SN1 crosses this line and the low-Src area is no longer reachable after Src activation (dotted line). B: In the full model, with the CSK negative feedback loop in place, we therefore observe another set of bifurcation points (SN3 and SN4). Sustained activation of Src is not possible to the left of SN3.

In order to establish an explanation for the mechanism in which CSK is assumed to be regulated, we have created a hypothetical, alternative version of our model, where Cbp is not involved and CSK is activated by direct Src phosphorylation. Fig. 6B shows the same bifurcation diagram for this alternative model. In this theoretical system the positions of the bifurcation points SN3 and SN4 are directly proportional to the total concentration of CSK. A two-fold increase in CSK would therefore shift the bistable area from \( k_2 \in [0.67, 1.3] \) to \([2.0, 4.4]\). This is in contrast to the translocation model, where the bifurcation points asymptotically approach a finite value and the same increase has a weaker impact on the position of the bistable range (\( k_2 \in [0.87, 1.74] \)). The theoretical model thus displays greater sensitivity to CSK variation than the translocation model.

Translocation has the advantage that regulation depends on two protein concentrations instead of only one. Alterations to one of these have a smaller impact than in the simple activation model. However, experimental findings suggest that Src is in fact sensitive to singular variations of CSK. We will consider some explanations for this discrepancy under Discussion and Conclusion.
**Prerequisites for bistability**

Existence of bistability in the Src system depends on a number of model parameters. Surprisingly, our simulations show that an increased \( \rho_{\text{src}} \) (i.e. activity of unphosphorylated Src) eliminates bistability (Fig. 7). For the default parameter set, the unstable branch disappears above \( \rho_{\text{src}} \approx 0.075 \). However, a reasonable separation of branches is only achieved below \( \rho_{\text{src}} \approx 0.02 \). This figure corresponds to at least a 50-fold increase of activity upon Tyr418 phosphorylation (\( \rho_{\text{src}}/\rho_{\text{src}} \)).

Due to autocatalytic activity it is difficult to experimentally correlate phosphorylation state of Tyr418 to Src activity. Under *in vitro* conditions autophosphorylation of wildtype Src cannot be eliminated completely. Kmiecik and Shalloway have used Src mutants possessing a phenyalanine substitution at Tyr418 (Y418F) to study the effects of autophosphorylation deficiency. Their *in vitro* kinase assays suggest a more modest, roughly five-fold increase in activity, corresponding to a \( \rho_{\text{src}} \) of 0.2. However, they also show that the Y418F mutant has drastically lowered *in vivo* activity (Kmiecik and Shalloway, 1987, Table 1). The small bistable area of Fig. 7A suggests that activity of the unphosphorylated kinase domain is in fact nearly or completely absent.

The model also allows for an alternative explanation, which is that pTyr418 is required for some targets, but not for others. Strictly speaking any enzymatic rate is dependent on both enzyme and substrate. Kinase assays are usually carried out with small detectable peptide substrates. It is conceivable that larger protein substrates behave differently and that various conformations of Src show diminished (or possibly even enhanced) activity towards those substrates. The central ‘Src activity’ node in our model admittedly is a simplification.

To further investigate this hypothesis we extended our model to account for substrate-specific Src activities. The extended model contains three different nodes that replace the ‘Src activity’ node: Cbp-specific, PTPr-specific and autophosphorylation-specific activity. The simulation results show that only \( \rho_{\text{src}} \) rates for PTPr-directed activity and autophosphorylation are critical for bistability. Above \( \rho_{\text{src},\text{auto}} \approx 0.12 \) the system becomes monostable, while variation of \( \rho_{\text{src,Cbp}} \) has no effect on stability (data not shown). In conclusion, our model suggests that the activity of Src in absence of Tyr418 phosphorylation is either entirely suppressed or at least ineffective on the two positive feedback loops.

**Role of the positive feedback loops**

Positive feedback is known to be an essential ingredient for bistability in biochemical systems (Cinquin and Demongeot, 2002; Angeli et al., 2004). The system described here provides a good example of sophisticated control over a positive feedback loop.

In fact, our model contains two positive feedback loops (see Fig. 1): Src autophosphorylation (B) and the PTPr loop (C). The strength of loop B is determined by the kinetic parameter \( k_3 \). Loop C depends on multiple parameters: \( k_2, K_{\text{act}} \) and \( k_{\text{PTPr}} \). Similar to \( k_2 \), \( K_{\text{act}} \) is a physiological parameter. \( K_{\text{act}} \) is directly proportional to the activity of serine/threonine kinases that phosphorylate PTPr, inhibiting the interaction of PTPr and Grb2. Because of its position in the model, the behaviour produced by variation of \( K_{\text{act}} \) is analogous to \( k_2 \).

As we have demonstrated in Fig. 5, the parameter \( k_2 \) is dependent on phosphorylation status of the N-terminal Src phosphorylation sites, which in turn are controlled by cdc2 activity. cdc2 effectively determines the amount of positive feedback that Src experiences after dephosphorylation on pTyr529. Thus, Fig. 5 gains a new interpretation: it demonstrates the influence of the external positive feedback loop (loop C) on Src activation and deactivation.

While decreasing \( k_2 \) confines bistability to a very small region, inhibiting Src-mediated tyrosine phosphorylation of PTPr has an even more dramatic effect on bistability (Fig. 7B). If \( k_{\text{PTPr}} \) falls below approx. 0.12, the bistable region disappears. Even an unreasonably large increase of \( k_3 \) – the parameter controlling feedback loop B – cannot reinstate bistability. The conclusion is that internal feedback is not sufficient to maintain bistability in the Src system.

**DISCUSSION AND CONCLUSION**

We have developed a dynamic systems model of a small set of interacting proteins centred around the protein tyrosine kinase Src. The model reproduces its activation during mitosis, as influenced by the tyrosine kinase CSK and tyrosine phosphatase PTPr. We have shown that the model is consistent with the observations made in normal, healthy cells, as well as in a number of perturbed systems such as CSK mutants.

The simulations reveal an interesting implementation of bistability at the base of an excitable system. Bistability is frequently associated with systems which display a sustained response to a short, transient signal, toggling between two discrete states. Excitable behaviour is less common in biochemical systems. The combination of these two elements produces a system capable of differentiated responses. Depending on the parameter \( k_3 \) we observe either full excitability, sustained Src activation or a bistable combination of both. Part of this complex behaviour may be required for biological phenomena such as cell cycle checkpoints.

If we accept bistability as an explanation for the complex manner in which Src is regulated, the model raises several questions. Bistability only exists if we assume that Src activity is completely or almost completely inactive under absence of phosphorylation of

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*Fig. 7. Critical parameters for existence of bistability. Graphs show deviations from default parameter set, where \( \rho_{\text{src}} = 0 \) and \( k_{\text{PTPr}} = 1 \). A: Participation of Cbp (i.e. not phosphorylated on Tyr418 nor Tyr529) in positive feedback removes bistability. B: External positive feedback via PTPr is required for bistability. Variation of \( k_2 \) and \( k_3 \) yields different limits, but the critical point remains significantly above zero within physiologically acceptable parameter ranges.*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Description</th>
</tr>
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<tr>
<td>( \rho_{\text{src}} )</td>
<td>0.2</td>
<td>Activity of unphosphorylated Src</td>
</tr>
<tr>
<td>( k_{\text{PTPr}} )</td>
<td>1</td>
<td>Kinetic parameter</td>
</tr>
<tr>
<td>( k_3 )</td>
<td>0.075</td>
<td>Kinetic parameter</td>
</tr>
<tr>
<td>( K_{\text{act}} )</td>
<td>0.02</td>
<td>Physiological parameter</td>
</tr>
</tbody>
</table>

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*Note:* The table and graph data are placeholders and should be replaced with actual values for the parameters described in the text.
Tyra18 in the activation loop. This must be true for at least PTP-directed activity and for autophosphorylation. Kinase assays using small peptide substrates are probably not conclusive in this case.

Our simulations confirm that Src regulation is sensitive to the amounts of Cbp and CSK in the cell, although we observe a remarkable level of robustness to CSK overexpression, which is not observed in vivo. This discrepancy could indicate a role for other regulatory components in the system. There are several possible explanations: Src not only resides at membranes, but it also participates in cytoplasmic interactions. Even if regulation of membrane-bound Src was not affected by CSK overexpression, the detrimental effect of overexpression on cytoplasmic Src could be responsible for the observed lethality. Also it is not clear whether Cbp is increased in cell lines that overexpress CSK. If this is the case, this could indicate a regulatory influence of CSK on the expression of Cbp. The observed dynamics would be similar to Fig. 6B. Unfortunately, there is little data available about the Cbp/CSK subsystem, for example regarding expression levels of Cbp in wildtype and CSK overexpression cell lines or about its deactivation by tyrosine phosphatases. The resolution of this problem requires experimental evidence.

There is currently little supporting evidence for the existence of feedback loop C (Fig. 1) involving PTPα. Our results show that a feedback loop of this kind is likely to exist. Together with bistability critical characteristics of the system behaviour are lost without it. For example, control exerted by cyclin-dependent kinases (such as cdc2) is dependent on external positive feedback. These issues, too, will need to be addressed experimentally.

The behaviour we have reproduced probably demonstrates only a small part of what the tyrosine kinase Src is capable of. For example, it is known to also reside in the cytoplasm, or at other membranes than the cytoplasmic. Other phosphorylation sites influence its interactions and activity. PTPα is known to form inactive dimers, which could constitute a negative regulatory mechanism (Jiang et al., 2000). Src interacts with numerous other kinases, phosphatases, and transcription factors. Its regulation in another context is bound to be different from what we have described here. In order to understand the purpose of Src and kinase signalling in general the possibilities in these domains will need detailed exploration.

REFERENCES


