FEBS Letters 589 (2015) 667–671

FEBS Letters

journal homepage: www.FEBSLetters.org

Hypothesis

Model scenarios for switch-like mitotic transitions

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ARTICLE INFO

Article history:
Received 11 December 2014
Revised 2 February 2015
Accepted 4 February 2015
Available online 12 February 2015

Edited by Ivan Sadowski

Keywords:
Biochemical kinetics
Greatwall-kinase
Phosphatase
Mitotic control
Bistability

ABSTRACT

To facilitate rapid accumulation of Cdk1-phosphorylated substrate proteins, the Cdk1 counter-acting phosphatase, PP2A-B55 is inhibited during M phase by stoichiometric inhibitors (ENSA and Arpp19). These inhibitors are activated when phosphorylated by Cdk1-activated Greatwall-kinase. Recent experiments show that ENSA is dephosphorylated and inactivated by the PP2A-B55 itself, and acts as an unfair substrate inhibiting PP2A-B55 activity towards other Cdk1 substrates. Mathematical modelling shows that this mutual antagonism between the phosphatase and its inhibitor is insufficient to explain the switch-like characteristics of mitotic entry and exit. We show that the feedback regulation of Greatwall activating kinase and/or inactivating phosphatase can explain the abruptness of these cell cycle transitions.

1. Introduction

Phosphorylation of proteins during mitosis requires that the activity of protein-kinases overcome their counter-acting protein-phosphatases. Therefore, mitotic phosphorylation is facilitated if the activation of the protein-kinase is accompanied by the simultaneous inhibition of its counter-acting phosphatase. The cyclin-dependent kinase-1 B-type cyclin complex (Cdk1:CycB) reduces the activity of some counter-acting phosphatases in mitosis [1,8,13,15]. For example, Cdk1:CycB phosphorylates Greatwall-kinase (Mastl in human), which in turn phosphorylates and thereby activates two small-molecular inhibitors of PP2A:B55 (Endosulfine and Arpp19, collectively referred here as ENSA) [3,9]. Phosphorylated ENSA (pENSA) binds to and inhibits PP2A:B55, thereby the BEG (PP2A:B55/ENSA/Greatwall) pathway facilitates the complete phosphorylation of certain Cdk1:CycB substrates at mitotic entry and their timely dephosphorylation at mitotic exit [2].

At the end of mitosis Cdk1 is inactivated by degradation of Cyclin-B, and inhibition of PP2A:B55 is relieved so that it dephosphorylates a subset of Cdk1 phospho-sites. Recent work has illustrated that the reactivation of PP2A:B55 depends on its own intrinsic activity. In the pENSA-phosphatase complex the phosphate group of ENSA can be only removed by the 'inhibited' PP2A:B55. Therefore pENSA is not only an inhibitor but also a substrate of the phosphatase by an 'unfair competition' mechanism [14]. This situation is reminiscent of the relationship of Cyclin-dependent kinases (Cdks) with their stoichiometric inhibitors p27 (human), Sic1 (budding yeast) and Rum1 (fission yeast), and of the Anaphase Promoting Complex/Cyclosome with the Mitotic Checkpoint Complex (MCC) [12]. In all of these cases the inhibitor binds to the corresponding enzyme and occupies the active site until the enzyme eventually inactivates it. The generality of this type of substrate-inhibitor relationship in cell-cycle control systems makes it important to analyse the dynamic consequences of such a mechanism.

Williams et al. [14] has provided a careful kinetic analysis of the pENSA-PP2A:B55 'unfair competition' mechanism. In this work, we extend their model by the relevant protein-kinases (Greatwall and Cdk1:CycB) whose activities might be regulated directly or indirectly by the PP2A:B55 phosphatase. The emerging interaction network of protein-kinases and- phosphatases traces out complex biochemical regulatory networks which are difficult to understand by simple intuitive reasoning. In order to overcome this difficulty we use mathematical tools of dynamical systems theory (rate plots and phase plane analyses) which help to guide our thinking about complex networks. Our work also shows that mathematical modelling provides a useful approach to predict the dynamic characteristics of biochemical networks, as illustrated by many previous studies [4,11]).

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http://dx.doi.org/10.1016/j.febslet.2015.02.007

2. Results

2.1. The substrate-inhibitor model for ENSA and PP2A:B55 interactions

In general terms, alternative substrates of an enzyme are competing for the same binding-site, and hence naturally 'inhibit' one other. The extent of this inhibition is proportional to the residence time of the enzyme:substrate complex. Dephosphorylation of pENSA by PP2A:B55 follows the standard Michaelis–Menten mechanism [14]:

$$\mathrm{pENSA} + \mathrm{B55} \underset{k_{\mathrm{diss}}}{\overset{k_{\mathrm{ass}}}{\leftrightarrows}} \mathrm{pENSA:B55} \overset{k_{\mathrm{cat}}}{\rightarrow} \mathrm{ENSA} + \mathrm{B55}$$

with very small catalytic rate constant ($k_{\mathrm{cat}}^{B55} = 0.05\ \mathrm{s}^{-1}$) and Michaelis-constant ($K_M^{B55} = 1$ nM). Importantly, these values are two and four orders magnitude smaller than corresponding parameters for dephosphorylation of Cdk1-phosphorylated substrates by PP2A:B55 [14]. Therefore pENSA titrates PP2A:B55 away from Cdk1 phosphorylated substrates, and thereby inhibits their dephosphorylation. However, pENSA is turned over continuously by PP2A:B55 because it is also a substrate of the phosphatase.

Within the cell, ENSA is in stoichiometric excess ($[ENSA]_T \cong 1000$ nM) over the phosphatase ($[B55]_T \cong 250$ nM) [14], fulfilling a necessary requirement for this type of inhibition. $[ENSA]_T$ and $[B55]_T$ are distributed among the following forms:

$$[ENSA]_T = [ENSA] + [pENSA] + [pENSA:B55]$$

$$[B55]_T = [B55] + [pENSA:B55]$$

By introducing the sum of the ENSA phosphorylated forms ($[pENSA]_T = [pENSA] + [pENSA{:}B55]$) the kinetics of pENSA dephosphorylation can be described by an algebraic- and a differential-equation:

$$[pENSA:B55] = \frac{[pENSA]_T + [B55]_T + K_M^{B55} - \sqrt{\left([pENSA]_T + [B55]_T + K_M^{B55}\right)^2 - 4[pENSA]_T[B55]_T}}{2} \quad (1)$$

$$\frac{d[pENSA]_T}{dt} = -k_{\mathrm{cat}}^{B55}[pENSA:B55] \quad (2')$$

Eq. (1) is the solution of a quadratic equation derived from the assumption that the pENSA:B55 complex is in steady state:

$$K_M^{B55} = \frac{[pENSA][B55]}{[pENSA:B55]} = \frac{([pENSA]_T - [pENSA:B55])([B55]_T - [pENSA:B55])}{[pENSA:B55]}$$

The temporal dynamics of pENSA dephosphorylation is obtained by simultaneously solving Eqs. (1) and (2′) numerically (Fig. 1A), which re-capitulates the calculations of Williams et al. (see their Fig. 10A). This reveals that initially the rate of pENSA dephosphorylation is constant and the level of pENSA decreases linearly with time, because PP2A:B55 is saturated with pENSA. Once $[pENSA]_T$ drops below the level of the phosphatase ($[pENSA]_T/[ENSA]_T < 0.25$), free PP2A:B55 appears and the rate of pENSA dephosphorylation gradually declines, because not all PP2A:B55 is bound to pENSA. A rate-plot of pENSA dephosphorylation as a function of $[pENSA]_T$ (Fig. 1B, red curve) has two linear segments that meet where $[pENSA]_T$ becomes equimolar with total phosphatase ($[B55]_T/[ENSA]_T = 0.25$). The rate of pENSA dephosphorylation is linearly dependent on $[pENSA]_T$ when the phosphatase is not saturated with its 'inhibitory' substrate. In contrast, the rate is at its maximal constant value when pENSA_T is in stoichiometric excess over the phosphatase and the free PP2A:B55 becomes negligible.

In this form the substrate-inhibitor model simulates ENSA dephosphorylation at the end of mitosis in the complete absence of Greatwall-kinase activity (Fig. 1A). Although Greatwall-kinase loses its Cdk1-dependent activatory phosphorylation during the meta/anaphase transition, a scenario with zero kinase activity is not a biologically realistic assumption. Therefore in the next section, we test how sensitive the substrate-inhibitor mechanism is to pENSA production by Greatwall-kinase.

2.2. Closing the cycle by ENSA phosphorylation

We supplement Eq. (2′) with a term for Gw dependent phosphorylation of ENSA:

$$\frac{d[pENSA]_T}{dt} = k'_{pGw}[pGw]([ENSA]_T - [pENSA]_T) - k_{\mathrm{cat}}^{B55}[pENSA:B55] \quad (2)$$

where k'_{pGw} and $([ENSA]_T - [pENSA]_T)$ represent the efficiency ($k_{\mathrm{cat}}^{Gw}/K_M^{Gw}$) of phosphorylated (active) Gw-kinase (pGw) and the concentration of unphosphorylated ENSA, respectively. The rate of ENSA phosphorylation defines a straight line on the rate plot (Fig. 2, red lines) with a slope proportional to the activity of Greatwall. The rate of phosphorylation is linear with substrate concentration ($[ENSA]_T - [pENSA]_T$) because the Michaelis-constant for Greatwall (K_M^{Gw}) is assumed in the μM range characteristic for typical protein-kinases [7]. Wherever the two curves intersect, the rate of phosphorylation (red curves) and dephosphorylation (green curves) are balanced and $[pENSA]_T$ comes to a steady state. PP2A:B55 is fully active only if the two rate curves intersect near the origin for k'_{pGw} $[pGw]$ value equal to zero. Higher Greatwall activities move the steady state to higher $[pENSA]_T$ levels and thereby decrease free PP2A:B55. Since the bulk of the phosphatase is inactivated when a quarter of ENSA_T is phosphorylated, free PP2A:B55 becomes negligible when the activity of Greatwall ($k'_{pGw}[pGw]$) becomes one third ($0.0166\ \mathrm{min}^{-1}$) of PP2A:B55 activity ($0.05\ \mathrm{min}^{-1}$).Greatwall-kinase is activated by Cdk1 phosphorylation and inactivated by dephosphorylation. The following differential-equation for phosphorylated Greatwall (pGW) assumes that pGW is dephosphorylated by an unidentified, constitutive phosphatase (PPX):

$$\frac{d[pGw]_T}{dt} = k'_{Cdk}[Cdk]([Gw]_T - [pGw]) - k'_{ppx}[pGw] \quad (3)$$

where k'_{Cdk} and k'_{ppx} are the efficiencies ($k_{\mathrm{cat}}/K_{\mathrm{M}}$) of Cdk1 and PPX phosphatase. With this equation we assume that the cellular Greatwall concentration is below the typical Michaelis-constant (10–100 μM) of Cdk1 and PPX [7,14]. Eqs. (1)–(3) allow us to calculate the steady state response of free PP2A:B55 and pENSA_T levels to Cdk1 activity (Fig. 3). The pENSA-PP2A:B55 substrate-inhibitor mechanism gives a proportional response to Cdk1 activity in terms of PP2A:B55 inhibition. We conclude that the unidirectional BEG pathway from Cdk1 to PP2A:B55 operates as a **rheostat** similar to the volume control on a radio.

In *Xenopus* cell free extracts the BEG pathway shows a switch-like response during interphase and M phase transitions [9]. In interphase, both ENSA and Greatwall are dephosphorylated and PP2A:B55 is active. In contrast, ENSA becomes abruptly phosphorylated in M phase by activated Greatwall-kinase and inhibits PP2A:B55 [9]. Furthermore, experiments suggest that the Cdk1 thresholds for BEG activation and inactivation are different, which calls for a hysteresis effect. In human cells, PP2A:B55 is only re-activated in late mitosis after anaphase onset when Cdk1 activity becomes inhibited by separase [2], suggesting that the