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Review

Bistability by multiple phosphorylation of regulatory proteins

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ABSTRACT

The activity of a protein can be reversibly modulated by post-translational, covalent modifications, such as phosphorylation and dephosphorylation. In many cases, the modulated protein may be phosphorylated by the same kinase on many different amino acid residues. Such multisite phosphorylations may occur progressively (during a single binding event of kinase to substrate) or distributively (the kinase dissociates from its substrate after each phosphorylation reaction). If a protein is phosphorylated by a distributive multisite mechanism, then the net activity of a population of these protein molecules can be a highly nonlinear function of the ratio of activities of the kinase and phosphatase enzymes. If the multiply phosphorylated protein is embedded in a positive feedback loop with its kinase and/or phosphatase, then the network may exhibit robust bistable behavior. Using numerical simulations and bifurcation theory, we study the properties of a particular bistable reaction network motivated by the antagonistic relationship between cyclin-dependent kinase and its multiply phosphorylated target, Cdh1, which is involved in the degradation of cyclin molecules. We characterize the bistable switch in terms of (i) the mechanism of distributive phosphorylation (ordered or disordered), (ii) the number of phosphorylation sites on the target protein, (iii) the effect of phosphorylation on the target protein (abrupt or progressive inactivation), and (iv) the effects of stochastic fluctuations in small cells with limited numbers of kinase, phosphatase and target proteins.

Contents

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1. Introduction

Cellular proteins are often subject to post-translational modifications like phosphorylation (Manning et al., 2002), acetylation (Mellor, 2006), hydroxylation (Schofield and Ratcliffe, 2004) and ubiquitinylation (Hershko and Ciechanover, 1998). These covalent modifications are made on specific amino acid residues of the target protein by modifying enzymes (kinases, methylases, etc.). Usually the modifications are removed by another enzyme working in the opposite direction (phosphatases, demethylases, etc.). Often the same type of modification happens at more than one amino acid of the target protein, a phenomena called multisite modification.

Post-translational modifications change the biochemical properties of the target protein. For example, the phosphorylation of a transcription factor may exclude it from the nucleus (see e.g., Moll et al., 1991). In this way, reversible covalent modification of proteins provides a fast and efficient way to regulate protein functions, and it is used to control all basic cellular processes, including metabolism, signaling, motility, growth, proliferation, differentiation, organelle trafficking and membrane transport.

The most characteristic example of reversible, multisite protein modification is phosphorylation and dephosphorylation by protein kinases and phosphatases. Like any other multisite modification, multisite phosphorylation can happen via a processive or distributive mechanism (Gunawardena, 2005). During processive phosphorylation, the kinase phosphorylates more than one amino acid residue on its substrate during a single binding event. Therefore the kinetics of a processive mechanism is not essentially different from a single phosphorylation event. In contrast, under the distributive rule, only one phosphorylation takes place during a single enzyme–substrate binding event. After the substrate is phosphorylated, the kinase dissociates from its substrate and then must bind anew at a different site of phosphorylation. A distributive mechanism is said to be disordered or ordered if the kinase phosphorylates the target sites randomly or in a specific sequence, respectively. Of course, the mechanism of dephosphorylation may also be either processive or distributive (ordered or disordered).

Because multiple binding events occur during a distributive mechanism, a sigmoidal signal–response curve was expected between the phosphorylation state of the target protein (the response) and the ratio of kinase activity to phosphatase activity (the signal) (Ferrell, 1996). However, an analytical study of the ordered distributive case revealed a more complex story (Gunawardena, 2005). The fraction of the target protein in the maximally phosphorylated form (f_N) is maintained close to zero below a threshold (θ) of the kinase/phosphatase ratio (K_T/H_T). The value of θ increases with the number of phosphorylation sites N. Close to the threshold, f_N increases abruptly from 0 to 0.5. Above the threshold, f_N increases gradually (hyperbolically) from 0.5 to 1 with increasing K_T/H_T. Therefore multisite phosphorylation makes a good threshold, but it is a poor switch (in Gunawardena's terminology).

Our interest in multisite phosphorylation stems from the fact that many cell-cycle regulatory proteins are multiply phosphorylated by cyclin-dependent kinase (CDK). Examples include Wee1 (Kim and Ferrell, 2007; Mueller et al., 1995), Cdc25 (Kumagai and Dunphy, 1992), Sic1 (Verma et al., 1997) and Cdh1 (Zachariae et al., 1998). During progression through the cell cycle, these proteins undergo characteristic shifts, easily visible on polyacrylamide gels, between their unphosphorylated and heavily phosphorylated forms. The possible roles of multisite phosphorylation of these proteins have been considered in several papers from Qu's group (Qu et al., 2003; Yang et al., 2004). They showed that multiple phosphorylation of regulatory proteins like Cdc25 and Sic1 can produce threshold response curves (Cdc25 activity as a function of CDK activity). When these proteins are coupled in positive and negative loops back to CDK, the networks generate interesting emergent behavior, such as bistability and limit cycle oscillations.

To understand and extend this pioneering work, we undertook a close analysis of the properties of multisite phosphorylation chains in isolation and embedded in positive feedback loops. In this paper, we will review how simple mass-action kinetics of elementary phosphorylation events can generate highly nonlinear signal–response curves (f_N versus K_T/H_T) when the target is distributively phosphorylated on multiple sites. Then we use these results to study the emergent properties of bistability and hysteresis in the double-negative feedback loop between CDK and Cdh1. This example is of great significance for cell cycle regulation (Tyson and Novak, 2008). Of course, similar examples could be given from other aspects of cell physiology (metabolism, motility, etc.) and for other sorts of covalent modifications (methylation, hydroxylation, etc.). In all cases, the basic principles are the same.

2. Results

2.1. Steady-state distribution of phosphoforms

Let us consider a protein X with N phosphorylation sites, and let XP_0, XP_1, …, XP_N refer to the different phosphorylated forms. For simplicity, we assume that the sum of the concentrations of all forms of X is constant: $[XP_0] + [XP_1] + \ldots + [XP_N] = X_T = \text{constant}$. We assume that the phosphorylation and dephosphorylation reactions are distributive. Let k and h denote the activities of the kinase and phosphatase, respectively (activity = probability of an event per unit time per unit concentration of substrate). We assume that these activities are independent of the phosphorylation state of the substrate, XP_i.

For an ordered distributive mechanism (Fig. 1A, top), the net phosphorylation rate is the same for each step of the chain, and the same is true of the net dephosphorylation rate. Assuming that these rates are limited by the binding of enzyme to substrate, we write $v_{phos} = k_{assoc}[K][XP_i]$ and $v_{dephos} = h_{assoc}[H][XP_i]$. If the enzyme–substrate complexes are so short-lived that their concentrations can be neglected compared to K_T and H_T, then we can assume that $v_{phos} = k[XP_i]$ and $v_{dephos} = h[XP_i]$, where $k = k_{assoc}K_T$ and $h = h_{assoc}H_T$.

For a disordered mechanism (Fig. 1B, top), the rates of phosphorylation and dephosphorylation depend on the number of unphosphorylated and phosphorylated sites, respectively, on the substrate. With the same assumptions in the previous paragraph, the rates of phosphorylation and dephosphorylation of XP_i are given by $(N-i)k[XP_i]$ and $ih[XP_i]$, respectively.

If kinase and phosphatase activities are constant in time, the phosphorylation chain will approach a steady state in which the concentrations of the various phosphorylated forms no longer change with time. For an ordered distributive mechanism, the steady-state concentrations are given by

$$\frac{[XP_i]}{X_T} = \frac{\left(\frac{k}{h}\right)^i}{\sum_{j=0}^{N}\left(\frac{k}{h}\right)^j} = \frac{\left(\frac{k}{h}\right)^i\left(1-\frac{k}{h}\right)}{1-\left(\frac{k}{h}\right)^{N+1}}. \quad (1)$$

These fractions are plotted as functions of k/h in Fig. 1A (bottom). At low and high values of k/h, the dominant species are the unphosphorylated and the fully phosphorylated forms, respectively. When $k = h$, the different phosphoforms are uniformly distributed.

For a disordered mechanism, the steady-state concentrations of phosphoforms are given by (Salazar and Hofer, 2007):