Multisite Protein Phosphorylation as a Molecular Timing Device

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***Short Abstract* — In recent years, a great number of regulatory processes has been found to involve multisite phosphorylation. It dramatically increases the possibilities for phosphorylation states ($\leq 2^N$ for N sites) and phosphorylation routes ($\leq$ N!). We developed an elementary-step formalism for the regulation of multisite protein phosphorylation. We found that the order in which the individual residues are phosphorylated is of overriding importance for both sensitivity and speed of response. Our analysis also suggests a general role for multiple phosphorylation in the synchronization of molecular events, in particular, for the coherent initiation of DNA replication at several replication origins.**

I. INTRODUCTION

MULTISITE protein phosphorylation is a common regulatory mechanism in cell signaling. Compared to the phosphorylation of a single residue, multisite phosphorylation dramatically increases the possibilities for protein-protein interactions, conformational regulation, and phosphorylation routes [1,2]. Multisite phosphorylation has been also implicated in the precise timing of diverse cellular processes such as cell-cycle progression.

Here we provide a mathematical model for the regulation of multisite protein phosphorylation based on the mechanistic description of elementary steps [1]. We analyze how the order of phosphate processing modulates the sensitivity and kinetics of response and how this can contribute to the precise timing of molecular events.

II. RESULTS

A. Order of phosphate processing

Target proteins may differ in the order of phosphate processing. A particular site can be modified irrespective of the phosphorylation state of the other sites. Alternatively, the enzyme can process their target sites in a strictly ordered sequence. A sequential order reduces the number of phosphoforms from 2^N in the random mechanism to N+1, where N is the number of phosphorylation sites.

We found that the dose-response curve of the fully phosphorylated substrate is modulated by the order of phosphorylation. Sequential processing is characterized by steeper response curves. A random mechanism gives rise to shallow response curves, favoring intermediate phosphorylation states. Because the number of intermediate states grows exponentially with random order and only linearly with sequential order, the difference in the steepness of the response curve becomes more pronounced when the number of phosphorylation sites is large.

B. Phosphorylation kinetics

Upon a change in kinase or phosphatase activities, a target protein reaches a new phosphorylation state after certain transition time elapsed. For a sequential mechanism, the transition time to fully phosphorylate the substrate exhibits a maximum when kinase and phosphatase activities balance. By contrast, the phosphorylation kinetics for a random mechanism depends on the enzyme activity in the same way as a single-site phosphorylation. Multisite phosphorylation is achieved much faster by a random mechanism than by a sequential one because there are many more phosphorylation routes available for the random mechanism.

C. Synchronization of molecular events

Multisite phosphorylation may control the synchronization of molecular events such as the firing of several DNA replication origins during the cell cycle. Coherence of origin firing depends on the kinetics of two branches that converge at the origins: (i) multiple phosphorylations leading to the formation of a complex activator outside the origins, and (ii) the assembly of the replicative complex at the origins. We found that a mixed random-sequential mechanism of phosphorylation allows a delayed and pronounced sigmoidal kinetics for the assembly of the complex activator. This ensures the previous assembly of the replicative complex and a precise timing of origin firing. Our model show that firing is more coherent the larger the number of phosphorylation steps and similar the steps will be.

III. CONCLUSION

Multisite phosphorylation may serve as a precise timing device that ensures the synchronization of molecular events. The particular order of phosphorylation is essential for this.

REFERENCES

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