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Review

Processive phosphorylation: Mechanism and biological importance ☆

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Abstract

Recent proteomic data indicate that a majority of the phosphorylated proteins in a eucaryotic cell contain multiple sites of phosphorylation. In many signaling events, a single kinase phosphorylates multiple sites on a target protein. Processive phosphorylation occurs when a protein kinase binds once to a substrate and phosphorylates all of the available sites before dissociating. In this review, we discuss examples of processive phosphorylation by serine/threonine kinases and tyrosine kinases. We describe current experimental approaches for distinguishing processive from non-processive phosphorylation. Finally, we contrast the biological situations that are suited to regulation by processive and non-processive phosphorylation.

Keywords: Processive phosphorylation; Serine/threonine kinase; Tyrosine kinase; Posttranslational modification; Cas; SR proteins

Contents

1. Introduction

1.1. Multisite protein modification

The inventory of proteins in a eucaryotic cell is increased significantly by covalent modifications to amino acid sidechains

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or to the polypeptide backbone [1]. These posttranslational modifications represent the most common mechanism by which the functions of proteins can be altered. In many cases, multiple modifications (of the same chemical type or of different types) can occur on a single polypeptide, giving rise to a complex regulatory network that controls protein function. The multisite modification of such proteins has been referred to as a "molecular barcode." According to this hypothesis, each combination of modifications on a protein conveys a unique meaning that can be interpreted by the cell [2,3].

The barcode hypothesis was first advanced in the context of histone modification. The posttranslational modification of histone proteins plays an important role in regulating heterochromatin-mediated silencing, chromosome segregation, and gene expression [3–8]. The N-terminal tails of the four core histone proteins (H2A, H2B, H3, and H4) have multiple sites that undergo lysine acetylation, lysine methylation, serine phosphorylation, and other types of covalent modification. Separate enzymes exist to catalyze the addition and removal of these modifications; thus, the modification pattern is dynamic and responsive to the status of the cell. The patterns of histone modifications are believed to guide the interactions between histones, chromatin, and non-histone regulatory proteins. Histone acetylation and methylation have recently been studied on a genomic scale [3,9].

The p53 tumor suppressor also undergoes a complex set of modifications. In response to various genotoxic stresses such as DNA damage, nucleotide depletion, and hypoxia, p53 accumulates in the nucleus and undergoes activation, leading to growth arrest or apoptosis. The p53 protein is covalently modified on multiple sites at its N-terminus and C-terminus. The predominant modification at the N-terminus is phosphorylation, whereas the C-terminal modifications include phosphorylation, acetylation and sumoylation [10]. A variety of protein kinases have been shown to target p53, including DNA-activated protein kinase, members of the casein kinase family, mitogen-activated protein kinases (MAPKs), stress activated protein kinases, and protein kinase C [11,12]. Cell signaling pathways that converge on p53 result in dynamic changes to the pattern of modification.

1.2. Multisite phosphorylation

Approximately one-third of all eucaryotic proteins are modified by phosphorylation on serine, threonine, or tyrosine during their lifetime in the cell. These phosphorylation events control a multitude of cellular functions [1,13–15]. It is increasingly clear that the activities of many proteins are regulated by phosphorylation at more than one site. In a recent study using a mass spectrometric approach to identify and quantitate phosphorylation sites as a function of stimulus, time, and subcellular location, it was shown that a majority of proteins detected in response to stimulation with epidermal growth factor (EGF) were phosphorylated on multiple sites [16]. Of the proteins containing a phosphorylation site that responded to EGF, most (77%) contained an additional site that responded differently; thus, protein phosphorylation often plays distinct roles at different sites on a protein.

The stoichiometry of multisite phosphorylation can be remarkably high. The C-terminal repeat domain (CTD) of RNA polymerase II undergoes extensive phosphorylation and thereby serves as a binding scaffold for a variety of nuclear factors [17–19]. The CTD comprises approximately 25 to 52 tandem copies of the consensus repeat heptad YSPTSPS [20]. The CTD contains as many as 50 phosphorylated residues *in vivo* [18]. The predominant phosphorylation near the beginning of genes occurs on Ser5, whereas phosphorylation of Ser2 predominates near the end of genes [21]. This phosphorylation pattern is crucial in order to determine which factors associate with RNA Pol II.

In some cases of multisite phosphorylation, the sheer number of sites appears to be more important than the identities of the individual phosphorylated residues. Multisite phosphorylation of the yeast cyclin dependent kinase inhibitor Sic1 sets a threshold for the onset of DNA replication. Out of the nine possible phosphorylation sites on Sic1, six or more must be phosphorylated to promote binding to the ubiquitin ligase SCF^{Cdc4}, which then targets Sic1 for degradation [22,23]. Strikingly, phosphorylation of any combination of six sites (but not five or fewer) appears to be sufficient for SCF^{Cdc4} binding. In another example, multisite phosphorylation of Ste5, a MAPK scaffold protein, is essential for inhibition of the yeast mating pheromone pathway. These multiple phosphorylations (on eight Ser/Thr sites) inhibit membrane binding (and pheromone signaling) by repelling the negatively charged lipids of the inner leaflet of the plasma membrane. Thus, multisite phosphorylation of Ste5 provides a switch-like deactivation that is driven by bulk electrostatics [24].

1.3. Multisite phosphorylation by two or more kinases

There are numerous cases in which phosphorylation of a protein at one site increases its activity, while phosphorylation at a second site (by a second kinase) decreases its activity. For example, the activity of the cell cycle regulator Cdc25 is governed by antagonistic phosphorylation events. Cdc25 is a protein phosphatase that removes the inhibitory phosphates on Cdc2, thereby triggering entry into mitosis [25]. Phosphorylation of Ser216 on Cdc25 is catalyzed by the checkpoint control kinases CHK1 and CHK2 in response to DNA damage. Phosphorylation at Ser216 promotes binding to 14-3-3 proteins and cell cycle arrest at the G2/M checkpoint [26,27]. In contrast, in the transition to mitosis, Cdc25 is phosphorylated at several alternate sites by the Cdc2/cyclin B complex (Thr48, Thr67, Ser122, Thr130, and Ser214) [28,29]. These phosphorylations activate Cdc25, contributing to a feedback activation loop that facilitates the rapid onset of mitosis [25].

The activities of G-protein coupled receptors (GPCRs) are regulated by multiple Ser/Thr protein kinases. Agonist-induced desensitization of GPCRs involves phosphorylation of the intracellular portions of the receptors by G-protein coupled receptor kinases (GRKs), cAMP-dependent protein kinase (PKA), or protein kinase C (PKC) [30]. The GRK family consists