Review

When Worlds Collide—Mechanisms at the Interface between Phosphorylation and Ubiquitination

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Abstract

Phosphorylation and ubiquitination are pervasive post-translational modifications that impact all processes inside eukaryotic cells. The role of each modification has been studied for decades, and functional interplay between the two has long been demonstrated and even more widely postulated. However, our understanding of the molecular features that allow phosphorylation to control protein ubiquitination and ubiquitin to control phosphorylation has only recently begun to build. Here, we review examples of regulation between ubiquitination and phosphorylation, aiming to describe mechanisms at the molecular level. In general, these examples illustrate phosphorylation as a versatile switch throughout ubiquitination pathways, and ubiquitination primarily impacting kinase signalling in a more emphatic manner through scaffolding or degradation. Examples of regulation between these two processes are likely to grow even further as advances in molecular biology, proteomics, and computation allow a system-level understanding of signalling. Many new cases could involve similar principles to those described here, but the extensive co-regulation of these two systems leaves no doubt that they still have many surprises in store.

Post-translational modifications (PTMs) are an essential element of functional biological systems. Amongst PTMs, phosphorylation catalysed by protein kinases and ubiquitination catalysed by ubiquitin ligases are amongst the most prevalent. Both reversible covalent modifications have demonstrated roles in altering the function, binding partners, and localisation of proteins, and their abundance makes it inevitable that the two exert both positive and negative regulation on each other.

Protein kinases are amongst the most well-studied signalling enzymes in the eukaryotic cell. The human kinome is made up of more than 500 proteins, which phosphorylate a diverse range of substrates [1]. Cumulative knowledge from structural studies has allowed a general model of kinase regulation to be developed around the catalytic and regulatory spines common to most characterised kinases [2,3]. Kinases are often organised in auto-regulatory cascades to provide robust response and strong signal amplification to various stimuli [4–6]. Although the core enzymatic architecture of protein kinases is similar, the mechanisms that control key regulatory elements such as the DFG motif at the base of the activation loop and the regulatory α-C helix are significantly more diverse. Mechanisms differ markedly between kinases and even upon the regulation of the same kinase by different stimuli.

While regulation of protein ubiquitination has attracted attention similar to phosphorylation, the inherent diversity of the ubiquitin system means that more varied mechanisms are involved [7,8]. Ubiquitin is attached via a three-step cascade, whereby the first two steps are common, and the third conjugation step diverges markedly. Initially, an E1 enzyme acyl-adenylates the C terminus of ubiquitin and forms a covalent bond between the active site cysteine and the C-terminal glycine of ubiquitin[9]. Ubiquitin is then

transferred via a transthiolation reaction to the active site of an E2 (ubiquitin-conjugating) enzyme [10]. From here, the major classes of E3 ubiquitin ligases transfer ubiquitin to substrates via one of three major mechanisms: (i) RING E3 ligases stabilise a "closed" conformation of the E2-Ubiquitin conjugate, priming the thioester bond between E2 and ubiquitin for attack by a lysine residue from the substrate; (ii) HECT E3 ligases themselves attack the thioester bond and form a covalent HECT–Ub complex that is then attacked by a substrate lysine residue; or (iii) RING-between-RING (RBR) ligases utilise a RING domain but employ a distinct mechanism to form a RBR–Ub covalent complex that is subsequently attacked by a substrate lysine. Ubiquitin ligases are subject to intense self-regulation, be it via autoubiquitination or ubiquitination by another E3 ligase [11]. These modifications can be non-proteolytic, both activating and deactivating, or proteolytic [12–15]. Enzymes from all three classes of ubiquitin ligases are known to be regulated by phosphorylation, with RING and RBR ligase regulation described in structural detail in this review.

In direct opposition to ubiquitin ligases, deubiquitinases (DUBs) are ubiquitin-specific proteases (USPs) that cleave the isopeptide or peptide bond either between ubiquitin and substrate or between ubiquitin moieties in a polyubiquitin chain. They are essential components of the ubiquitin system, processing ubiquitin precursors to their mature form, keeping free ubiquitin levels high, and providing signal termination in the absence of stimulus or rescue from degradation [16–18]. They are sometimes found in complex with E3 ligases, fine-tuning the extent of ubiquitination or editing the present ubiquitin code [19,20].

One clear avenue for ubiquitination to regulate kinase signalling is the attachment of degradative ubiquitin chains to kinases in order to tag them for proteasomal degradation. Although this is clearly an important example of interplay between the two systems, it is conceptually relatively straightforward. Here, we aim to review the more nuanced structural mechanisms of interplay between the enzymes that confer these two classes of PTM. While previous reviews have addressed this crosstalk [21], in recent times, structural studies have provided elegant atomic level mechanisms across various systems. These examples include control of ubiquitin ligase substrate recognition through phosphorylation specific motifs, direct activation of ubiquitin ligases by phosphorylation, regulation of kinase activity by conjugated ubiquitin or scaffolding ubiquitin chains, and phosphorylation-specific regulation of deubiquitinating proteases. While not exhaustive, we hope that these examples describe concepts that may extend more widely, particularly as proteomic methods allow more in-depth study of crosstalk between the two prevalent systems [22].

Phosphodegrons—The Marker before the Marker for Degradation

Diverse classes of ubiquitin E3 ligases perform the attachment of ubiquitin to substrates, including RING, HECT, and RBR-type ligases. Amongst RING-type ligases, the Cullin E3 ligases comprise a major subclass, whose modular nature enables an impressive array of ubiquitinating machineries. Functional aspects of Cullin ligases are described in detail elsewhere [23], but in short, they are assembled around one Cullin family member (of which there are seven) and the Skp1 adapter. The Cullin protein recruits a RING domain, and Skp1 recruits a substrate receptor, often in the form of an "F-box" containing protein [hence termed Skp1-Cullin-F-box (SCF) ligases]. One of these F-box proteins, β-TrCP1, provides one of the earliest examples of phosphorylation-specific protein ubiquitination [24], often termed phosphodegron recognition.

β-TrCP1 consists of an F-box and a WD40 substrate recognition region. The β-TrCP1 F-box protein preferentially recruits substrates that have been doubly phosphorylated on a DpSGΦXpS motif. This motif is found within β-catenin and IκB, amongst other proteins [25]. The structure of phosphodegron from β-catenin bound to β-TrCP1 was solved more than a decade ago [24] and showed that the β-TrCP1 WD40 domain uses the top face of its β-propeller to recruit the phosphorylated substrate peptide, which spans the central cleft. Phosphorylated serine residues bind to either side of the cleft, forming hydrogen bonds with several polar residues and nearby arginine residues that are integral to the interaction. The model derived from this work was that increased substrate binding affinity generated through phosphorylated residues acts to hold β-catenin in close proximity of the Cullin RING domain, which recruits ubiquitin-conjugated E2 enzyme. In this way, substrate lysine residues from β-catenin are well placed to attack the ubiquitin–E2 thioester bond during ubiquitination.

Subsequent structures of the related phosphospecific F-box protein, Fbw7, bound to a phosphorylated Cyclin E substrate showed an extremely similar mechanism of phosphodegron recognition [26]. This work also offered the additional insight that Fbw7 is able to form dimers, as can its yeast homologue, Cdc4. The presence of tandem substrate recruitment domains due to dimerisation offers the possibility for another layer of regulation, bearing in mind that the substrates of Fbw7 and Cdc4 are known to contain multiple phosphodegrons. More recent mechanistic and structural studies of Cdc4 have explored this phenomenon further. Tang *et al.* demonstrated that weak binding by combinations of four potential phosphodegrons are essential for tuning the threshold of substrate degradation by Cdc4 [27]. Even though