Phosphorylation Meets Proteolysis

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Phosphorylation is a reversible post-translational modification that regulates many proteins and enzymes, including proteases, as shown by two recent publications. Huang and colleagues and Velázquez-Delgado and Hardy (this issue of Structure) describe how phosphorylation activates the protease activity of the deubiquitinizing enzyme DUBA and how it inhibits caspase-6, respectively.

Post-translational protein modification through phosphorylation is central to the regulation of key cellular processes. The human “kinome” (Manning et al., 2002; http://kinase.com/human/kinome/), consisting of at least 518 different kinases, catalyzes millions of distinct phosphorylation events. Another well studies post-translational event is proteolysis, which is catalyzed by members of the human “degradome” (http://degradome.uniovi.es/dindex.html). It is well appreciated that so-called limited proteolysis, where a specific protease cleaves a specific substrate at one or several specific sites, leading to its activation, deactivation, or subcellular relocation rather than its complete degradation, is implicated in many cellular events ranging from cell division (Dephoure et al., 2008) to apoptosis (Kurokawa and Kornbluth, 2009). Although the two events are fundamentally different—phosphorylation is a reversible modification, whereas proteolysis is irreversible—there is mounting evidence that kinases and proteases work hand-in-hand. For example, the regulation of both cell proliferation and apoptosis is dependent on the interplay between protease and kinases (López-Otin and Hunter, 2010). Caspase-3-dependent processing and consequent inactivation of the serine-threonine protein kinase AKT1 turns off survival pathways, whereas some deubiquitinating proteases (DUBs) stabilize kinases by removing ubiquitin tags from proteins otherwise destined for degradation by the ubiquitin-proteasome pathway. Likewise, phosphorylation is known to activate or inactivate proteases. Although it is somehow easier to rationalize how a proteolytic event leads to the activation or inactivation of a functional protein, the structural changes induced by phosphorylation are more subtle. Two recent papers provide exciting insight into how phosphorylation can directly regulate protease activity. While Huang et al. (2012) provide a structural explanation of how phosphorylation activates the deubiquitinating protease DUBA, Velázquez-Delgado and Hardy (2012; this issue of Structure) show that introducing a mutation that mimics a biological phosphorylation event inactivates the apoptotic protease caspase-6.

The deubiquitinating activity of human deubiquitinase DUBA is strictly dependent on the phosphorylation of Ser177 by the casein kinase II (CK2). Indeed, the ligand-free, unphosphorylated DUBA rests in an inactive state, as the substrate binding site is misaligned and parts of the molecule appear to be highly mobile. By itself, CK2 phosphorylation does not induce any structural changes that would be consistent with protease activation. The active conformation is induced only upon ubiquitin binding, as if the enzyme “folds around its substrate” (Huang et al., 2012). The phosphate group stabilizes the substrate-protease interaction but does not directly interact with the active site residues (Figure 1). It clamps together two helices and appears in turn to stabilize the core of the DUBA structure and is involved in direct interactions with C-terminal part of the ubiquitin substrate.

Substrate-induced activation has previously been observed for other, structurally diverse DUBs such as UCH-L3 and USP7 (Hu et al., 2002; Johnston et al., 1999). In their ligand free forms, these enzymes exist in an inactive resting state. Ubiquitin binding induces the maturation of the otherwise obstructed and misaligned substrate binding site and catalytic center. This substrate-dependent
activation mechanism might be the basis for the high specificity of deubiquitinating proteases; most of the enzymatically well-characterized DUBs are virtually inactive on short peptidic substrates but are highly efficient enzymes when cleaving ubiquitin based substrates (for UCH-L3, see Dang et al. [1998]). Phosphorylation as described for DUBA acts as a switch for deubiquitinating activity, thus adding another level of control.

The structural determinants of how phosphorylation can directly inhibit a protease are illustrated by the structure of the caspase-6 mutant Ser257Asp reported by Velázquez-Delgado and Hardy (2012). This mutation mimics the phosphorylation of Ser257, which is catalyzed by the kinase ARK5. In the structure of wild-type caspase-6, Ser257 is located more than 10 Å away from the primary specificity pocket and does not directly interact with the substrate or the catalytic center. Steric bulk introduced by phosphorylation leads to rearrangement of loops around the catalytic center and the substrate recognition groove. These structural changes have limited impact on the catalytic residues but inactivate the enzyme by obliterating the substrate recognition site.

Although phosphorylation impacts protease activity of DUBA and caspase-6 in opposite ways, both modifications appear to regulate protease activity by impacting substrate recognition rather than catalysis. In the unphosphorylated and catalytically inactive state of DUBA, the catalytic center is in an active conformation and the phosphate group linked to Ser177 is required to align the substrate binding site and substrate. Likewise, phosphorylation of caspase-6 deforms the substrate recognition site, but the catalytic dyad again appears to be in a catalytically competent conformation. This observation suggests that alignment and misalignment of the substrate binding site, rather than alteration of the catalytic residues, is a common strategy for regulation of protease activity. In effect, this is an analogous mechanism to that of trypsinogen activation. The Ser-His-Asp catalytic triad of trypsigen, the inactive form of the digestive protease trypsin, adopts a conformation almost identical to that observed in the mature enzyme (Bode et al., 1978). Upon cleavage of its propeptide, the novel N-terminus of trypsin forms a buried, intramolecular salt bridge, thereby forming the oxyanion hole (required to stabilize the high-energy intermediate during catalysis) and rigidifying the highly mobile "activation domain", including critical sections of substrate-binding pocket. In contrast to phosphorylation though, this is an irreversible event, and trypsin activity can only be ablated by endogenous inhibitors. Phosphorylation can potentially provide a much more nuanced strategy for modulation of signaling pathways where proteases, kinases, and phosphatases play a critical role.

Furthermore, the structures of caspase-6 and DUBA also underscore the inherent plasticity of these enzymes. More and more structures indicate that proteases adopt multiple conformations and do not simply rest as an inactivezymogen that is then transformed directly into the active form during maturation. More precisely, maturation is a multistep process that includes spatial and/or temporal regulation, post-translational modification, and substrate-induced conformational modification and allows for fine tuning of the proteolytic signal. Using small molecules or biologics to trap these enzymes in an active or inactive state could also allow for more precise control of dysregulated biological processes and is an attractive avenue for the development of pharmacological intervention (Lee and Craik, 2009).

REFERENCES


