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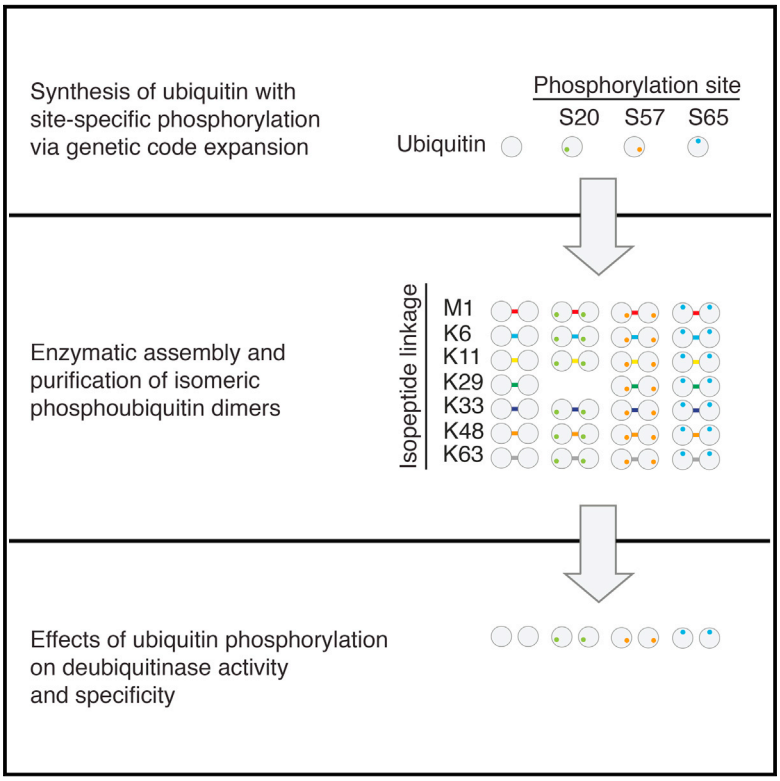
Synthesis of Isomeric Phosphoubiquitin Chains Reveals that Phosphorylation Controls Deubiquitinase Activity and Specificity

Graphical Abstract



Authors

Nicolas Huguenin-Dezot, Virginia De Cesare, Julien Peltier, ..., Yogesh Kulathu, Matthias Trost, Jason W. Chin

Correspondence

m.trost@dundee.ac.uk (M.T.), chin@mrc-lmb.cam.ac.uk (J.W.C.)

In Brief

Huguenin-Dezot et al. combine genetic code expansion and enzymatic assembly to synthesize 20 isomeric phosphoubiquitin chains with distinct Ser phosphorylation and/or isopeptide linkage sites. They discover that ubiquitin phosphorylation can control E3 ligase specificity and deubiquitinase specificity.

Highlights

- Milligram quantities of ubiquitin phosphorylated at Ser 20, 57, or 65 are purified
- Twenty isomeric phosphoubiquitin dimers are assembled and purified
- UBE3C chain synthesis specificity is controlled by Ser 20 ubiquitin phosphorylation
- Phosphorylation of ubiquitin controls deubiquitinase activity and linkage specificity

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Synthesis of Isomeric Phosphoubiquitin Chains Reveals that Phosphorylation Controls Deubiquitinase Activity and Specificity

Nicolas Huguenin-Dezot,[1] Virginia De Cesare,[2] Julien Peltier,[2] Axel Knebel,[2] Yosua Adi Kristaryianto,[2] Daniel T. Rogerson,[1] Yogesh Kulathu,[2] Matthias Trost,[2,*] and Jason W. Chin[1,*]
[1]Medical Research Council Laboratory of Molecular Biology, Francis Crick Avenue, CB2 OQH Cambridge, UK
[2]Medical Research Council Protein Phosphorylation and Ubiquitylation Unit, University of Dundee, DD1 5EH Dundee, UK
*Correspondence: m.trost@dundee.ac.uk (M.T.), chin@mrc-lmb.cam.ac.uk (J.W.C.)
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SUMMARY

Ubiquitin is post-translationally modified by phosphorylation at several sites, but the consequences of these modifications are largely unknown. Here, we synthesize multi-milligram quantities of ubiquitin phosphorylated at serine 20, serine 57, and serine 65 via genetic code expansion. We use these phosphoubiquitins for the enzymatic assembly of 20 isomeric phosphoubiquitin dimers, with different sites of isopeptide linkage and/or phosphorylation. We discover that phosphorylation of serine 20 on ubiquitin converts UBE3C from a dual-specificity E3 ligase into a ligase that primarily synthesizes K48 chains. We profile the activity of 31 deubiquitinases on the isomeric phosphoubiquitin dimers in 837 reactions, and we discover that phosphorylation at distinct sites in ubiquitin can activate or repress cleavage of a particular linkage by deubiquitinases and that phosphorylation at a single site in ubiquitin can control the specificity of deubiquitinases for distinct ubiquitin linkages.

INTRODUCTION

The post-translational modification of proteins with ubiquitin (Ub) modulates an expanding array of cellular processes (Komander and Rape, 2012). Ub is attached to a target protein through the formation of an isopeptide bond between a lysine in the target protein and the C terminus of the Ub. A cascade of enzymes (E1s, E2s, and E3s) directs protein ubiquitination: Ub is first activated, as a thioester conjugate to a cysteine residue in an E1-activating enzyme, and is then transferred to the active site cysteine of an E2-conjugating enzyme, before it is conjugated to the target protein with the help of an E3 ligase. The E3 may either activate direct transfer of Ub from the E2 (RING and U-box E3 ligases), or it may transiently accept Ub from the E2 to form an E3-Ub intermediate before transfer of Ub to the target amine (RBR and HECT E3 ligases) (Berndsen and Wolberger, 2014). In human cells there are two E1s, ~40 E2s, and >600 E3s (Clague et al., 2015).

The action of the E1, E2, and E3 cascade is counteracted by deubiquitinases (DUBs) that cleave the isopeptide bond between the target protein and Ub. There are five established families of DUBs: Ub-specific proteases (USPs), Ub C-terminal hydrolases (UCHs), ovarian tumor proteases (OTUs), Josephins, and JAMMs (Komander et al., 2009). The interplay between the specificity of the E1, E2, and E3 systems and the specificity of DUBs defines the ubiquitinated proteome, and factors that alter the activity and specificity of these enzymes reconfigure the ubiquitinated proteome and alter biological outcomes.

Many proteins have been identified as targets of ubiquitination. Proteins can be mono-ubiquitinated or modified with Ub polymers, in which Ub is linked via an isopeptide bond between the C terminus of one monomer and a lysine residue (K6, K11, K27, K29, K33, K48, or K63) or via a peptide bond to the N terminus (M1) of another monomer.

Ub linkages have been identified in cells at varying abundances (Kulathu and Komander, 2012). Because of the low abundance of Ub chains (Kaiser et al., 2011) and the challenge of purifying specific Ub linkage isomers from cells, the development of methods for the synthesis of well-defined Ub chains (Faggiano et al., 2016; Kumar et al., 2010; Virdee et al., 2010) has been crucial for characterizing the structural and biochemical properties of Ub chains. The characterization of atypical Ub chains has often preceded an understanding of their physiological significance, and biochemical and structural data for Ub chains of unknown function continue to inform in vivo experiments aimed at addressing physiological significance (Bremm et al., 2010; Hospenthal et al., 2013; Kristariyanto et al., 2015; Michel et al., 2015).

Ub is a target for post-translational modifications, including acetylation (Ohtake et al., 2015) and phosphorylation (Kane et al., 2014; Kazlauskaite et al., 2014; Koyano et al., 2014). Several sites of phosphorylation (Thr7, Thr12, Thr14, Ser20, Ser57, Tyr59, Ser65, and Thr66) have been identified on Ub within mammalian cells (Herhaus and Dikic, 2015).

We recently reported the directed evolution of SepRS/$tRNA_{CUA}$ pairs (Rogerson et al., 2015) that function with a mutant of EF-Tu (Park et al., 2011) for the efficient genetic encoding of

