https://doi.org/10.1038/s41594-024-01240-7

Towards an improved understanding of ubiquitylation

First discovered more than five decades ago, protein ubiquitylation has proven to be an omnipresent post-translational modification regulating virtually every eukaryotic cellular process. With novel clinical applications and recent studies demonstrating ubiquitylation of biomolecules other than proteins, the interest in ubiquitin will not waver any time soon.

Ubiquitin is a small, globular protein, almost identical from yeast to human. Thanks to its ability to target different biomolecules, including itself, which creates polyubiquitin chains of different architecture and behaviors, it can generate diverse signals[1] that are decoded by designated readers in a context-specific manner. This broad signal versatility, illustrated in our cover by the colorful, traditional worry beads, allows ubiquitylation to regulate fundamental cellular processes, from protein stability and activity to subcellular localization and molecular interactions.

Ubiquitin conjugation to target biomolecules occurs via an enzymatic cascade, which entails ubiquitin-activating enzymes (E1s) relaying an activated ubiquitin to conjugating enzymes (E2s). E2s in turn collaborate with ubiquitin ligases (E3s) to tag specific targets with either multiple ubiquitin moieties forming chains or a single ubiquitin at a single or multiple residues. Notably, cells contain hundreds of E3 ligases, belonging to different families[2], such as RINGs (and their subfamily CRLs), HECTs and RBRs, which use diverse catalytic mechanisms to confer substrate specificity.

The original observations showing that protein ubiquitylation targets substrates for proteasomal degradation were recognized by the 2004 Nobel Prize in Chemistry. Since then, the field has vastly expanded in numerous directions, with ubiquitylation arising as a master regulator of autophagy[3] and the DNA damage response[4], exerting key roles in signaling in cell-autonomous immunity[5] and in controlling development[6]. Needless to say, *Nature Structural & Molecular Biology* (*NSMB*) has always been interested[7] in state-of-the-art studies that progress our mechanistic understanding of protein ubiquitylation and its cellular roles.

Firmly established as a degradative signal, ubiquitylation has emerged to have numerous additional cellular functions and potential therapeutic applications. In this issue of *NSMB*, we feature primary research that expands our fundamental understanding of writers and readers of ubiquitylation and degradation mechanisms, along with thoughtful pieces on how the field has evolved and its future directions.

Given their key role in dictating which substrates are ubiquitylated, we first emphasize the several novel studies we are publishing on ubiquitylation writers. Authors from the labs of Brenda Schulman and Gary Kleiger elucidate the molecular underpinnings of the rapid and highly specific-for-K48-chains reactions mediated by UBE2R-family E2s. They show that extraordinary catalytic efficiency is achieved by an E2 'synergy loop' connecting the CRL E3, donor and acceptor ubiquitins. Landmark structural work from the lab of Sonja Lorenz not only provides one of the first high-resolution structures of a HECT-type ligase, HACE1, but also delineates the dimerization-induced autoinhibition of HACE1 and the selectivity of the active ligase monomer for its substrate, GTP-bound RAC1. The lab of Satpal Virdee discovers a unique 'hemiRING' zinc finger in the giant E3 ligase UBR4, a key regulator of protein degradation in neurons, and characterizes the molecular determinants in its specific pairing with UBE2A/UBE2B. A study from the Xing Liu lab shows that CAND1, a master regulator of CRLs, increases the dissociation rate of CRL2s and thus inhibits CRL2-dependent ubiquitylation, introducing an elegant mechanism that paces CRL2-mediated protein degradation. Finally, authors from the labs of Zhenguo Chen, Bruce Beutler and Lei Sun unveil the entire catalytic cycle, from assembly and substrate recruitment to (de)neddylation and CAND1-mediated substrate receptor exchange of $CRL3^{KBTBD2}$-dependent degradation of p85α, a key factor in PI3Kα-mediated signaling.

Furthermore, ubiquitin has shown itself to have many other cellular tricks up its sleeve. Context-dependent readers of the many different ubiquitylation flavors are the key interpreters of these elaborate signals. Work from the Man Pan and Lei Liu labs showcases the importance of specificity in recognizing ubiquitylated chromatin. By elucidating how the fusion oncoprotein SS18–SSX1 uses an unorthodox mechanism to bind ubiquitylated nucleosomes, the authors link the aberrant tethering of the chromatin-opening BAF1 complex to polycomb-repressed regions in synovial carcinoma. Further reflecting on how ubiquitylation is not all about protein degradation, a Historical Perspective from Rahman and Wolberger contextualizes how much we have learned since the original identification of protein ubiquitylation and highlights the paramount cellular importance of non-degradative polyubiquitylation, particularly in regulating the chromatin architecture and the interplay between different histone modifications.

One of the most promising developments in the field has been the potential to harness ubiquitin as a degradation tool in clinical applications[8]. These valuable efforts by both academia and industry have given rise to the field of targeted protein degradation (TPD), through molecular glues and proteolysis-targeting chimeras (PROTACs), and have led to the development of U.S. Food and Drug Administration (FDA)-approved drugs against tumors[9]. In a study from the Arvinas-based Békés lab, the authors expand the TPD arsenal by showing that the E3 ligase KLHDC2 can be co-opted by small molecules to proteolytically target several clinically relevant substrates. Reflecting on this cutting-edge field, Whelan and Mayor-Ruiz author a Comment on the status of the TPD field and note the importance of developing in silico and chemical tools to inform the rational design of novel TPD tools.

In what figures to be an exciting field of research in the upcoming years, recent work (reviewed in ref. 10) has unexpectedly

introduced the existence of non-protein ubiquitylation. In another insightful Comment, Lechtenberg and Komander survey the increasing number of non-canonical ubiquitylation events identified and emphasize the need to develop novel experimental tools to better understand the physiological importance of these events. Further contemplating on non-protein ubiquitylation, Noburu Mizushima contextualizes the roles of ubiquitylation of both traditional protein substrates and non-traditional non-protein targets in autophagy.

As these recent discoveries have shown, there is probably still so much to learn about this unique signaling molecule. In between following the progress of TPD in clinical applications, and understanding the physiological importance of non-protein ubiquitylation, we here at *NSMB* are eagerly looking forward to reading future insightful studies and hope to continue to provide a venue for pioneering ubiquitylation work that advances the field.

Published online: 16 February 2024

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