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EDITORIAL

Recent progress in ubiquitin and ubiquitin-like protein (Ubl) signaling

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Ubiquitin (Ub), a 76-residue polypeptide that is present in all eukaryotes, can be covalently conjugated to other proteins or other Ub molecules. Such ubiquitination (or ubiquitylation) reactions involve a cascade of reactions catalyzed by E1 (ubiquitin-activating enzyme), E2 (ubiquitin-conjugating enzyme) and E3 (ubiquitin ligase) proteins. The human Ub system encompasses ~40 E2s and ~700 E3s that specifically recognize and conjugate Ub to their substrates, including other Ub molecules. Protein ubiquitination is reversed by deubiquitinating enzymes (DUBs), which number close to 100 in humans. Ub can be ubiquitinated on any of its seven lysine residues or its N-terminus, leading to the formation of polyubiquitin chains in various configurations, each thought to create distinct signals recognized by specific Ub receptors. It has become clear that the functional consequences of protein ubiquitination can be far more diverse than simply tagging the protein for proteolytic elimination, although this is a frequent outcome. The term "ubiquitin signaling" collectively refers to the many ways that ubiquitination and deubiquitination reactions impact the function, localization, trafficking, or stability of a protein in the context of cellular regulation.

Bioinformatic, genetic and biochemical studies have unearthed a diverse set of Ub-like proteins (Ubls) that also covalently modify other proteins (and, in at least one case, lipids). These modifiers, like Ub, are activated and transferred to their targets by paralogous sets of E1-like, E2-like, and E3-like enzymes. The modifications also tend to be transient, being reversed by Ubl-specific proteases (ULPs). Consideration of the Ubls as part of the "Ub signaling" realm creates an even more intricate picture of how post-translational modification of proteins by other proteins can be exploited for the regulation of eukaryotic and, in some cases, prokaryotic cells.

Besides being modified by other Ub/Ubl molecules, Ub and Ubls are subject to further modifications, including phosphorylation, acetylation, and deamidation. This can profoundly affect the dynamics and specificity of the modifier, adding significant additional complexity to Ub signaling. Naturally, many research groups throughout the world have become fascinated by these emerging, unexpected facets of Ub signaling. Furthermore, inspired by the success of bortezomib, a proteasome inhibitor, in the treatment of patients with multiple myeloma, researchers are exploring the possibility of targeting various components of the Ub system for the development of therapeutics against different human maladies, including cancer, diabetes, and heart disease. In this special issue on "Ub and Ubl signaling", six papers contributed by experts in the field summarize exciting recent progress in our understanding of Ub and Ubl modifications and their contributions to cellular signaling mechanisms.

In their chapter, "Ubiquitin modifications", Swatek and Komander tackle the concept of a potential "Ub code." They first present an overview of known types of ubiquitin-protein modifications in cells and the distinct roles of specific polyUb chains in cellular signaling. They then focus on recent findings on novel modifications of Ub itself, such as acetylation and phosphorylation. With a focus on PINK1-mediated Ser65-phosphorylation of Ub and its role in mitophagy and activation of the Parkin ubiquitin ligase, they present a complex but intriguing picture of how phosphorylation of Ub, the basic module of the Ub signaling system, impacts cellular homeostasis and leads to distinct signaling outcomes.

As noted above, the human genome encodes ~40 Ub-conjugating enzymes (E2s) that play central roles in the transfer of Ub or Ubl proteins such as SUMO and Nedd8 to their targets. Despite normally being fairly small proteins, E2s dictate many aspects of the Ub signaling system, e.g., the efficiency of Ub chain assembly and specific configurations of different polyUb chains. In a review titled "E2 enzymes: more than just middle men", Klevit and Brzovic and their colleagues summarize functional and structural features that define unifying themes among the E2s and highlight emerging concepts in the mechanism and regulation of E2s. In particular, these authors provide a comprehensive view of how E2 activities are regulated through noncovalent and covalent interactions between E2s and other cellular components, including their cognate E3 ligases.

Much knowledge has been gained about how Ub/Ubl ligases specifically recognize their substrates and conjugate

them to Ub and Ubls. By comparison, the deconjugation of Ub and Ubls from their targets has been less thoroughly analyzed. In their article, "Substrate specificity of the ubiquitin and Ubl proteases", Ronau *et al.* review recent progress made on dissecting the molecular basis of substrate specificity of DUBs and ULPs, the enzymes that remove ubiquitin or Ubls from proteins and other molecules. By focusing on high-resolution structures of these specialized proteases, often as complexes with their substrates, their review explores how structural elements of each enzyme, and their rearrangement upon Ub/Ubl binding, help enhance substrate specificity. Lastly, by examining the often surprising substrate specificities of several non-eukaryotic DUBs and ULPs, the authors highlight the still considerable challenges of predicting the Ub and Ubl specificities of DUBs and ULPs.

In "Ub signaling in immune responses", Hu and Sun highlight some of the major functions of Ub signaling in regulating innate and adaptive immunity. Innate immune cells express several families of pattern-recognition receptors (PRRs) that mediate different immune responses to pathogen-associated molecular patterns (PAMPs). Indeed, a large number of E3 Ub ligases and DUBs act as important immune regulators that catalyze Ub conjugation and deconjugation of key components of the host immune system. The authors review the most recent findings about the dynamic and finely controlled ubiquitination that regulates immune functions. These range from the control of PRR signaling in innate immunity and inflammation to the activation and differentiation of immune cells. Ub-based regulation of central and peripheral immune tolerance to prevent autoimmunity is also considered.

Ubiquitination and timely degradation of cellular proteins is essential for maintaining cellular homeostasis. Dysregulated protein degradation is associated with many human diseases, including cancer and neurodegeneration. Therefore, targeting protein ubiquitylation and degradation represents a potential strategy to develop a wide range of therapeutics. In this special issue, Huang and Dixit summarize our current knowledge of druggable (and supposedly undruggable) targets in the Ub-signaling system and discuss the targeting strategies that are most promising. They consider the proteasome, Ub-ligating enzymes and DUBs as potential targets. Furthermore, these authors outline major challenges in trying to exploit the human Ub system for the development of novel therapeutics.

It has been known for some time that pathogenic bacteria express effector proteins that are secreted or injected into their eukaryotic hosts; some of these effectors bear a strong resemblance to components of eukaryotic Ub-signaling systems. In their review, Dikic and his colleagues focus on the "Bacteria-host relationship: ubiquitin ligases as weapons of invasion" and review recent progress in understanding the structures and functions of these "foreign" E3s. Having classified the bacterial E3s into three main classes, RING (really interesting new gene), HECT (homologous to the E6-AP carboxyl terminus) and NEL (novel E3 ligases), the authors summarize our current mechanistic understanding of these effectors and how they participate in host-microbe interactions important to infection and pathogenicity. The bacterially encoded E3s show remarkable diversity, and the authors emphasize the current lack of mechanistic understanding of a number of them. Finally, they discuss the technical challenges of, and potential solutions to, validating this class of enzymes as targets for the development of novel antibacterial therapies.

This special issue thus encompasses many exciting current areas of study on the structural, molecular and cellular biology of Ub/Ubl signaling. Needless to say, certain areas in the fast growing Ub/Ubl signaling field are not covered. It is our hope that readers, including those who may come from outside the research fields discussed here, will benefit from these expert perspectives. In this way, these reviews may entice newcomers to join in helping to advance this fascinating frontier.

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