Preface

The ability of the small polypeptide ubiquitin to be covalently attached to other intracellular eukaryotic proteins was discovered in the late 1970s and was initially most clearly linked mechanistically to protein degradation (Ciechanover, Heller, Elias, Haas, & Hershko, 1980; Ciechanover, Hod, & Hershko, 1978; Goldknopf & Busch, 1977). In the ensuing decades, an enormous range of physiological processes have been found to depend on ubiquitin–protein ligation (Varshavsky, 2017). Ubiquitination usually involves an amide linkage between the C-terminal carboxylate of ubiquitin and lysine side chains of the substrate protein (Pickart, 2001). Ubiquitin itself has seven lysines and a free amino group at its N-terminus. All of these amino groups can be coupled to additional ubiquitin molecules, leading to an elaborate array of potential ubiquitin polymer topologies and lengths that have different signaling functions (Swatek & Komander, 2016). For example, ubiquitin polymers bearing the first chain linkage ever described, involved ubiquitin Lys-48, were causally linked to recognition and degradation of the tagged protein by the proteasome (Chau et al., 1989).

Another remarkable development over the past quarter century has been the discovery of roughly a dozen other proteins that are structurally related to ubiquitin and also covalently modify cellular proteins (and, in at least one case, a lipid) (Hochstrasser, 2009). Thus, the "ubiquitin system" regulates an even wider range of processes than originally imagined. ISG15 was the first such ubiquitin-like protein (Ubl) to be discovered (Loeb & Haas, 1992), while the small ubiquitin-like modifier (SUMO) protein probably contributes the most varied Ubl–protein modifications other than those by ubiquitin itself. Both are known to be coupled to thousands of cellular proteins. The mechanistic consequences of attachment to the different Ubls are usually quite different from those of ubiquitination. Similarly, the physiological impact of these Ubl modifications, as with ubiquitin, is extremely broad.

The study of protein modification by ubiquitin and Ubls is now a mature—and vast—field. Nevertheless, new and completely unanticipated findings continue to emerge. For example, many bacteria (and other parasites) are known to take up residence within eukaryotic cells, and they often avoid destruction by the host or promote their own propagation by manipulating the host ubiquitin/Ubl system. They can do this by secreting ubiquitin ligases or deubiquitinases into the cytoplasm or deploying

enzymes that modify and inactivate ubiquitin or components of the host ubiquitination machinery (Ribet & Cossart, 2018). Among the most startling discoveries regarding a role for ubiquitin in host–bacterial pathogen interactions has been the finding that the intracellular bacterium *Legionella pneumophila*, the cause of Legionnaires' disease, ligates ubiquitin to specific cellular proteins by an unprecedented mechanism that completely bypasses the usual E1–E2–E3 enzyme cascade and requires no ATP (Puvar, Luo, & Das, 2018) (see chapter by Luo and colleagues).

The chapters in this volume describe a wide range of methods to study various aspects of ubiquitin- and Ubl-protein modification. Detailed protocols are integrated with focused discussions of what is known about the biochemistry, structure, and/or function of the particular ubiquitin/Ubl modifications described in each chapter. Three broad areas are covered in this volume: (1) various aspects of protein modification by ubiquitin itself; (2) substrate modification by different Ubls; and (3) removal of ubiquitin and Ubl proteins from their substrates by specialized proteases, the deubiquitinating enzymes and Ubl-specific proteases.

In line with the immense range of approaches that have been used to study the ubiquitin system, the methodologies described in the ensuing chapters are also extremely diverse. Sophisticated genetic methods are described for functional analyses, particularly well realized using the yeast *Saccharomyces cerevisiae* as a model, but also now possible with new gene-editing methods in mammalian systems. The ability to purify proteins from recombinant sources and the development of clever chemical probes has made possible precisely defined in vitro systems for studying important enzymological features of the ubiquitin system. High-resolution structural analysis of both ubiquitin/Ubl-protein ligation and deconjugation factors has also accompanied the maturation of the ubiquitin field, and examples of how samples are generated for these methods, such as the reaction of activity-based probes with deubiquitinases, are described here as well.

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References

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