

Abstract

Proteolysis via the ubiquitin-proteasome system (UPS) is a complex and tightly regulated process that plays a major role in basic cellular pathways. Many intracellular proteins are covalently modified with ubiquitin (Ub) or ubiquitin-like proteins (SUMO, ISG15, NEDD8). These modifications determine both protein turnover and functional status, and thereby provide a complex regulation of the proteome. With the multitude of substrates targeted, abnormalities in the UPS pathway are often implicated in diseases. In this dissertation, my aim is to investigate the role of structural disorder of different classes of UPS-associated proteins, and particularly how basic and ubiquitous components of the system in higher eukaryotes are performing specialized roles on degradation pathway. The enzyme cascade responsible for covalently linking Ub to its substrates consists of E1 (activating), E2 (conjugating), and E3 (ligase) activities. Eukaryotes have approximately 40 E2 and 600 E3 enzymes: each E2 is capable of binding specifically to multiple E3s, and E3s in turn can target multiple substrates. Although the E2-E3-substrate combination is known to give rise to the specificity of the UPS system, its origin is unexplored. By collecting known UPS-specific substrates from literature and databases, my idea is to determine the structural and functional basis of specificity. Furthermore, an extension of this analysis is to propose a 'tripartite' degron architecture of proteins where at least three distinct substrate elements (degrons) are used for promoting UPS-mediated degradation of a normal (functional) protein. My approach to study these features is to look into sequences, interactomes and structural data to get an overall view of UPS-mediated substrate degradation. My next goal is to address the question of predicting the likely substrates of specific E3s. This is approached by analyzing the sequence and structure-based interaction features of known E3-substrate pairs to generate a profile that characterizes E3 substrates, and then validate the prediction protocol developed.