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Review

Structural disorder and its role in proteasomal degradation

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ABSTRACT

The ubiquitin proteasome system is responsible for the controlled degradation of a vast number of intracellular proteins. It targets misfolded or otherwise aberrant proteins as well as proteins no longer needed at a given point in time. The 26S proteasome is a large macromolecular machine comprising 33 distinct subunits as well as a number of transiently associating cofactors. Being essentially a non-specific protease, specificity is conferred by the ubiquitin system, which selects and marks substrates for degradation. Here, we review our current understanding of the structure and function of the 26S proteasome; in doing so we highlight the role of disordered protein regions. Disordered segments in substrates promote their degradation, whereas low complexity regions prevent their proteolysis. In the 26S proteasome itself a main role of disordered segments seems to be rendering the ubiquitin receptors mobile, possibly supporting recruitment of polyubiquitylated substrates. Thus, these structural features of substrates as well as of the 26S proteasome itself likely play important roles at different stages of the protein degradation process.

1. Introduction

In eukaryotic cells the ubiquitin–proteasome system (UPS) is responsible for the regulated degradation of intracellular proteins [1]. Substrates of the UPS include proteins requiring tight temporal control, such as cell cycle regulators [2], as well as proteins that fail to pass the stringent quality control system of the cell [3]. The UPS has evolved to perform specific degradation of an extraordinarily broad range of substrates. Specificity is achieved by the covalent attachment of polyubiquitin chains to substrates via a cascade of E1/E2/E3 enzymes. The E3 enzymes in particular recognize primary 'degrons' of the substrate [4], often linear motifs embedded in disordered protein segments [5,6]. Polyubiquitin-tagged substrates are then recognized by a large multiprotein complex, the 26S proteasome, which degrades them into short peptides in an ATP-dependent manner [7].

Polyubiquitin tagging is required [8], but not sufficient for proteasomal degradation. For efficient degradation substrates must furthermore display unstructured stretches of minimal lengths at specific locations (Fig. 1). Jointly, the polyubiquitin tag and the unstructured region are sometimes referred to as a two-component secondary degron [9]. Conversely, tightly folded domains, such as dihydrofolate reductase (DHFR) bound to methotrexate can resist proteasomal degradation [10]. Moreover, specific types of low-complexity regions, most notably Gly-Ala repeats, also withstand degradation. This is exploited by some viruses, thereby escaping destruction [11,12].

The 26S proteasome comprises two subcomplexes, the 20S core particle (CP) and the 19S regulatory particle (RP) [13]. The CP is a fairly non-specific protease that catalyzes the degradation of unfolded substrates. Its crystal structures revealed a C_2-symmetrical assembly of 4 stacked heptameric rings [14–16]. The two central β-rings harbor the catalytically active sites sequestered in a catalytic chamber, whereas the outer ring α-subunits control access of proteins and peptides to it. The N-termini of the α-subunits form a gate at the cylinder openings of the CP [15,17,18].

One or both ends of the cylinder-shaped CP are capped by a 19S regulatory particle (RP), which prepares substrates for degradation [19]. The RP is a ~1 MDa complex consisting of 6 RP Triple-A (AAA) ATPases (Rpt1-6) and 13 RP Non-ATPases (Rpn1-3, 5-13, 15) (Fig. 2). The RP confers substrate specificity to the CP by recognizing polyubiquitylated substrates. Three integral, structurally distinct ubiquitin-receptors have been identified to date: Rpn10 [20], Rpn13 [21], and Rpn15 (Sem1) [22]. Additional functions of the RPs are the removal of polyubiquitin tags from the substrate prior to degradation by Rpn11 [23,24], and the ATP-dependent unfolding and translocation of the substrate into the CP by the Rpts [7,25–28].

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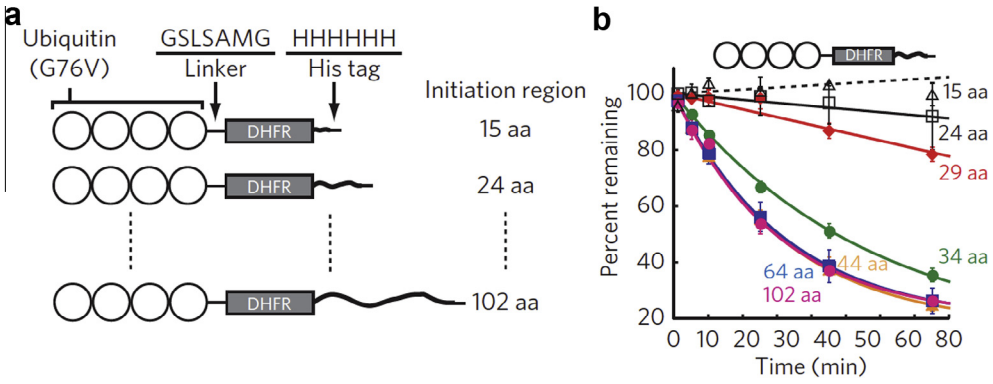

Fig. 1. Requirement of an unstructured initiation region for proteasomal degradation in vitro. (a) DHFR was targeted to the proteasome by N-terminal fusion with four ubiquitin moieties. Unstructured tails of different lengths were placed at the C-terminus to serve as initiation regions. (b) The substrate degradation rate strongly depends on the length of the unstructured tail, as indicated by the remaining percentage of the respective substrates over time. (Figure reproduced from [9].)

The structure of the 19S RPs is understood in less detail than that of the CP and only recently has its molecular architecture been elucidated primarily by single particle cryo-electron microscopy (cryo-EM) approaches [29–31]. The difficulties in structure determination arise from the structural and compositional heterogeneity of the 26S proteasome. This heterogeneity is due to structurally variable components of the RP, sub-stoichiometrical binding of many proteasome interacting proteins (PIPs), and conformational heterogeneity. Advances in cryo-EM in conjunction with powerful in vitro and in silico purification procedures have finally enabled subnanometer resolution insights into the 26S proteasome architecture and allowed to put forward an atomic model [29,32]. The structure reveals that the Rpts form homohexameric AAA-ATPase rings akin to prokaryotic ATP-dependent proteases, which associate with the cylinder ends of the CP and are surrounded by a shell of Rpn subunits. Subsequent studies have focused on capturing different conformational states of the 26S proteasome throughout its functional cycle [32–34]. These studies revealed structural changes of the AAA-ATPase module that translate into large motions of the Rpn subunits.

Here, we review our current understanding of the structure and function of the 26S proteasome with a focus on the role of variable and disordered protein regions. First we summarize the present knowledge with regard to the importance of disordered protein segments for their processing by the UPS. We then compile information of the structure of the RP and the most important PIPs including a prediction of their disordered segments using bioinformatics methods, before recapitulating the structure of the 26S proteasome in its different conformations and relating it to the sequence of events during degradation. Finally, we use image analysis methods to analyze structurally variable regions of the RP in cryo-EM data and compare them to the localization of unstructured elements.

2. Role of disordered regions in proteasomal substrates

For many substrates of the UPS, in particular those that underlie tight temporal regulation, their structures determine both, their ubiquitylation and proteasomal degradation. There is a variety of primary degrons recognized by E3 ligases, which induce the ubiquitylation of a protein [35]. Their structural determinants are best studied for the largest class of ligases, the Really Interesting New Gene (RING) ligases. Primary degrons for this type of ligases are typically linear motifs. In general, specificity-determining linear motifs themselves favor structural order, but they tend to be embedded in structurally disordered stretches of proteins [36]. The cullin RING ligases (CRLs) [5], which is the largest family of RING ligases, and the related Anaphase Promoting Complex/Cyclosome (APC/C) [6] indeed appear to recognize linear motifs embedded in unstructured segments. Degrons for APC/C are destruction (D) box and Lys-Glu-Asn (KEN) box motifs [37]. The KEN box is a preferred ubiquitin acceptor in APC/C substrates and the acceptor sites are enriched in predicted disordered regions [38]. This flexible design of substrate recognition and ubiquitylation sites allows for exquisite substrate specificity of ubiquitylation despite of the relatively low affinity of the D- and KEN-boxes through ‘processive affinity amplification’ [39]. The low affinity allows the APC/C to sample molecules in the cell quickly and the processive affinity amplification provides a positive feedback of secondary ubiquitylation reactions after conjugation of the first ubiquitin moieties. A further advantage of the embedding of primary degrons into unstructured segments may be the efficient regulation of its recognition by (de)-phosphorylation [40].

Accidental rather than intrinsic unfolding plays a key role in priming erroneous proteins for degradation by quality control pathways. These unfolded proteins bind to Hsp70 chaperones, which are in turn recruited to E3 ligases that ubiquitylate proteins resistant to folding [3]. The most prominent E3 ligases involved are the RING ligase carboxyl-terminus of protein (CHIP), which polyubiquitylates Hsp70 bound substrates in the cytosol [41] and the ER-resident RING ligase Hrd1 [42], which marks proteins for degradation that are detected by Kar2/BiP.

Disordered regions are also highly important for the kinetics of the proteasomal degradation process itself. Early hints at the features of substrates supporting or antagonizing protein degradation by the 26S proteasome came from the nuclear factor kappa-light-chain-enhancer of activated B cells (NFκ-B), one of the most intensely studied proteasomal substrates. The 26S proteasome is responsible for maturation of the p105 precursor into the active p50 transcription factor [43]. Thus, p105 is a good and a bad proteasomal substrate at the same time: its C-terminal domain is degraded efficiently, whereas its N-terminal p50 domain escapes degradation. Based on studies of the ER-membrane associated yeast homolog of p105, Spt23p p120, it was hypothesized that internal cleavage is initialized from an internal, unstructured region of at least 40 amino acids [44]. These unstructured residues were suggested to form a hairpin loop, entering the catalytic chamber of the CP. Subsequent bidirectional proteolysis of the polypeptides removes the C-terminal domain; the N-terminal domain escapes this fate due to its intrinsic properties [45].

The special case of NFκ-B motivated systematic analyses of in vitro degradation of chimeric constructs involving DHFR and disordered regions [46]. These studies revealed that unstructured stretches can indeed serve as degradation initiation sites and that

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