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Minireview

Stabilization signals: a novel regulatory mechanism in the ubiquitin/proteasome system

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Abstract The turnover of cellular proteins is a highly organized process that involves spatially and temporally regulated degradation by the ubiquitin/proteasome system. It is generally acknowledged that the specificity of the process is determined by constitutive or conditional protein domains, the degradation signals, that target the substrate for proteasomal degradation. In this review, we discuss a new type of regulatory domain: the stabilization signal. A model is proposed according to which protein half-lives are determined by the interplay of counteracting degradation and stabilization signals.

Key words: Degradation signal; Proteolysis; Neurodegeneration; Domains; Motifs

1. Introduction

It is not without good reasons that molecular biologists scrutinize novel proteins for functional domains in a first attempt to understand their contribution to the physiology of the cell. The presence of specific modular domains or motifs may give hints on protein localization, enzymatic activity, posttranslational modifications and interaction with other proteins. A clear illustration of the predictive power of domains can be found in the large variety of proteins involved in the ubiquitin/proteasome system. The presence of specific domains can reveal important functional features of these proteins such as their involvement in ubiquitination, characterized by the presence of HECT domains, RING fingers or U-boxes [1], their capacity to function as adaptor proteins, characterized by the presence of, for example, F-boxes [2], or their capacity to bind ubiquitin through UBA and UIM domains [3]. Not only the activity of proteins but also their lifespan is determined by the presence of specific domains or motifs. Of key importance are in this respect the degradation signals that target proteins for proteasomal degradation. In this minireview, we discuss recently published studies that suggest the presence of a second type of regulator of protein turnover, the stabilization signal, that counteracts the activity of degradation signals.

2. Degradation signals

The insight that the turnover of intracellular protein is a highly organized and energy-demanding process executed by the ubiquitin/proteasome system emerged only two decades ago. Since then, the combined effort of many researchers has unravealed a proteolytic machinery of unexpected complexity. At the basis of the proteolytic event lies a relatively simple two-step process: (1) proteins are provided with a degradation tag, a polyubiquitin tree consisting of multiple ubiquitin monomers, which is covalently linked by its carboxy terminus to the amino group of a lysine residue within the substrate [4]; (2) the polyubiquitinated substrate is recognized by a large proteolytic complex, the proteasome, which unfolds and progressively degrades the protein in small peptides [5]. These two core events are subject to a multitude of regulatory interactions that determine the selectivity and efficiency of ubiquitin/proteasome-dependent proteolysis.

A major determinant for protein half-life is the presence of degradation signals [6]. The first identified degradation signal, which regulates protein turnover by recognizing their N-terminal amino acid residue, was described shortly after the discovery of the ubiquitin/proteasome system [7]. This so-called N-end rule degron was soon followed by other well-defined degradation signals such as the ubiquitin fusion degradation (UFD) signal, the PEST sequence and the destruction box [6]. Some degradation signals were shown to be constitutive while others behave as conditional signals regulated by phosphorylation/dephosphorylation [8]. Both modular domains as well as small peptide sequences have been shown to act degradation signals. Regardless of the type of signal, each of these domains accelerates the turnover of proteins by recruiting an ubiquitin ligase, the specific E3 that conjugates a polyubiquitin tree to the substrate [1,4]. The efficacy of ubiquitin ligase recruitment is a major determinant of the ubiquitination rate. In several cases it has been shown that the degradation signal can be transferred to a different protein, which then becomes a substrate for the relevant ubiquitin ligase.

3. Stabilization signals

Recent evidence suggests that, in addition to degradation

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Abbreviations: EBNA1, Epstein–Barr virus nuclear antigen 1; GAr, Gly-Ala repeat; GRR, Gly rich region; UFD, ubiquitin fusion degradation

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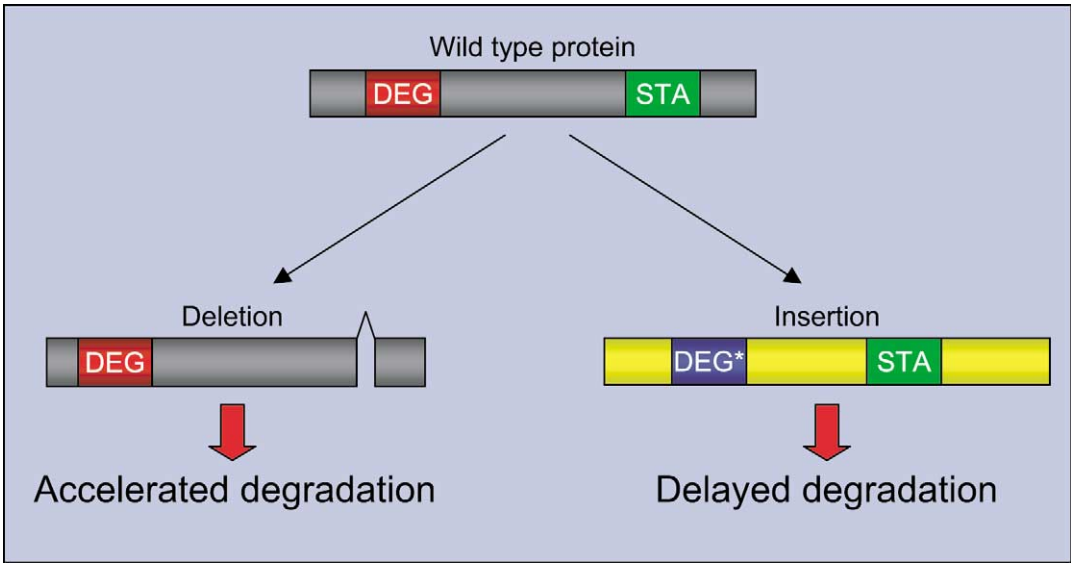

Fig. 1. Identification of stabilization signals. Stabilization signals can be revealed by a two-step experimental approach. Deletion of the suspected stabilization domain (STA) from the protein should result in accelerated degradation. Degradation should be accomplished through an endogenous degradation signal (DEG) that is also active in the wild-type protein and not through introduction of new degradation signals (for example, due to misfolding). Insertion of the stabilization signal into an unrelated proteasome substrate carrying a different degradation signal (DEG*) should delay or block degradation of the new host protein.

signals, proteins may also contain stabilization signals that act as positive regulators of protein half-life by delaying or blocking their degradation. In order to be classified as stabilization signals, these structures should share at least some of the properties of modular domains such as: (a) deletion of the domain from the wild-type protein should abrogate the phenotype associated with the domain and (b) introduction of the domain in a new host protein should results in a gain-of-function. When translated to the stabilization domain this means that their removal should results in accelerated turnover of the protein, while introduction of the domain in an unrelated proteasome substrate should slow down the turnover of the host protein (Fig. 1).

It is noteworthy that while degradation signals appear to act primarily by regulating the rate of ubiquitination, several events are potential targets for stabilization signals. For ex-

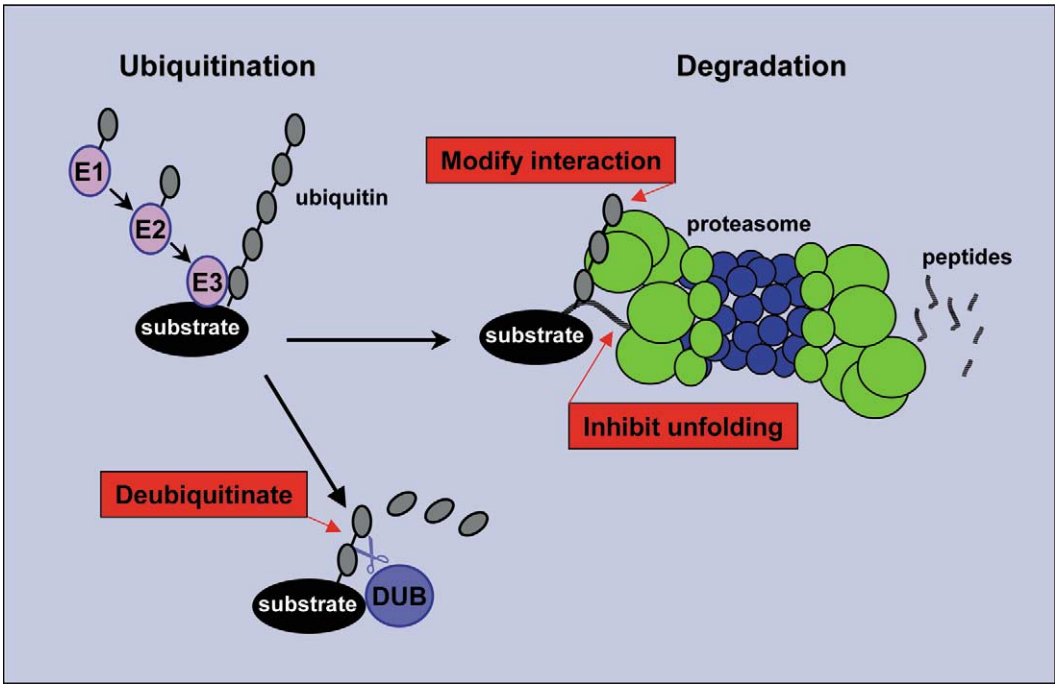

Fig. 2. Events in the ubiquitin/proteasome system that can be modified by stabilization signals. Substrates are targeted for proteasomal degradation by conjugation of a polyubiquitin tree through the concerted action of ubiquitin activase (E1), ubiquitin conjugases (E2) and ubiquitin ligases (E3). The polyubiquitinated substrate is bound to the proteasome and progressively degraded into small peptides. Stabilization signals can modify several discrete steps of the process including: (1) accelerate deubiquitination, (2) modify the kinetics of substrate–proteasome interaction or (3) block the unfolding of the substrate. DUB, deubiquitination enzyme.

ample, proteasomal degradation may be negatively regulated by accelerating the deubiquitination of substrates, by modifying the substrate–proteasome interaction or by hampering substrate unfolding. Hence, it may be possible to distinguish multiple classes of stabilization signals affecting different stages of the ubiquitin/proteasome system (Fig. 2). Three classes of stabilization signals that are supported by available experimental evidence will be discusses in the next section. Given the complexity of the ubiquitin/proteasome system, it is unlikely that these three classes will cover the full spectrum of possible stabilization signals.

3.1. Repetitive and low-complexity stabilization signals

The small proteolytic fragments that are produced by the proteasome are the major source of peptides that are presented at the cell surface by major histocompatibility class I molecules [9]. If the presented peptide is derived from a foreign or mutant protein this is likely to trigger specific cytotoxic T cells that will eliminate the altered cells. Viruses and other intracellular pathogens have evolved ways to interfere with antigen presentation [10]. The Epstein–Barr virus nuclear antigen 1 (EBNA1) contains a repetitive sequence that blocks proteasomal degradation [11,12]. The repetitive sequence varies in length in different viral isolates and consists exclusively of Gly and Ala residues. Deletion of the Gly-Ala repeat (GAr) restored proteasomal degradation of EBNA1 [12], while insertion of the GAr in an array of other proteasome substrates resulted in partial or full blockade of proteasomal degradation [13–15]. Moreover, the EBNA1 homologues encoded by related viruses that infect baboons and rhesus macaques contain similar repetitive sequences composed of Gly, Ala and Ser residues [16]. Although these repeats do not fully block antigen presentation, they do slow down the proteasomal degradation of the EBNA1 homologues. The mode of action of the GAr is not fully understood but several findings point to interference with a late step in the proteolytic process, possibly downstream of the interaction of ubiquitinated substrates with the proteasome. Of note, GAr containing proteins are ubiquitinatinated in vivo and can still interact with one of the proteasome subunits responsible polyubiquitin binding [15].

Interestingly, low-complexity sequences are required for partial processing of a few proteasome substrates. Presence of these sequences causes selective degradation of the part of the protein situated C-terminal to the sequence while the N-terminal domain and the low-complexity sequence itself are protected from degradation. The first example of this type of regulator was found in the p105 precursor of the transcription factor NF-κB which contains a Gly rich region (GRR) that is required for processing to the active p50 [17]. This sequence shows some similarity to the GAr and contains Ala residues that are crucial for its activity as a processing signal [18]. Importantly, it was shown that the GRR is not only required for processing of p105 but also prolongs the half-life of the resulting p50 [18]. Attempts to transfer this processing signal to other proteins have failed so far suggesting that additional peptide motifs may be required. More recently, it has been shown that two transmembrane transcription factors involved in the lipid metabolism of budding yeast, Spt23p and Mga2p, require processing in order to be released from the membrane [19]. Spt23p and Mga2 contain low-complexity domains at approximately the same position as the GRR and it has been postulated, though not proven, that these domains are functional analogues to the p105 GRR [20].

While additional studies are required to elucidate whether the viral GAr and the processing signals display a similar mode of action, the evidence for each of these low-complexity sequences suggests that they affect events downstream of the interaction with the proteasome providing some support for a shared inhibitory mechanism.

3.2. Conformational stabilization signals

Unfolding of the substrate by the regulatory subunit of the proteasome is an important step of ubiquitin/proteasome-dependent proteolysis. In two independent studies with designed proteasome substrates, degradation was inhibited by the introduction of tightly folded domains that resist the unfoldase activity of the proteasome [21,22]. In the first study, the introduction of two domains that fold upon binding their specific ligands caused a ligand-dependent stabilization of a proteasome substrate [21]. More recently, Navon and Goldberg have shown that the degradation of a proteasomal substrate was abrogated by linkage of a large biotin–avidin complex [22]. Interestingly in both studies a strong positional effect was observed suggesting that the proteasome initiates unfolding from a specific location within the protein. Furthermore, in the latter study the unfolding-resistant substrate functioned as general inhibitor of the ubiquitin/proteasome system. The biological significance of these observations based on designed substrates is supported by the study by Lee and co-workers indicating that, in addition to the GRR, protein folding may be important for the processing of p105 [21].

Increasing evidence implicates protein folding as a key determinant in the pathogenesis of neurodegenerative disorders that are caused by expansions of polyglutamine repeats in various proteins [23]. The expanded polyglutamine repeats were shown to slow down proteasomal degradation [24]. The characteristic formation of aggregates may partly explain this resistance as the tight complexes may hinder the unfolding of the proteins by the proteasome. Indeed, we have recently obtained evidence that the stabilizing effect of the expanded polyglutamine repeats is related to their capacity to form aggregates. While soluble polyglutamine containing substrates were rapidly degraded by the proteasome, their aggregated forms resisted proteasomal degradation [43]. Interestingly, similar to the designed unfolding-resistant substrate also the polyglutamine aggregates can cause impairment of the ubiquitin/proteasome system [25].

It is also noteworthy that an aberrant ubiquitin found in affected neurons of Alzheimer's patients is targeted for proteasomal degradation through a UFD signal but, when overexpressed, it is stable and functions as a general inhibitor of the ubiquitin/proteasome system [26,27]. Thus, the aberrant ubiquitin may act by challenging the unfolding or translocation activity of the proteasome.

3.3. Deubiquitination stabilization signals

The identification and characterization of enzymes that recognize and ubiquitinate the substrates has been a major focus in the ubiquitin/proteasome field [1]. While the pivotal role of ubiquitinating enzymes is undisputed, the interest in their enzymatic opposites, the deubiquitinating enzymes, is rapidly emerging [28]. The presence of a large number of deubiquitinating enzymes that share little sequence similarities suggests

a yet largely unexplored substrate specificity of this class of proteins [29].

Protein domains that recruit deubiquitinating enzymes and delay thereby the ubiquitin-dependent turnover of substrates are the most striking counterparts of the degradation signals. Surprisingly the knowledge about substrates of the large family of deubiquitinating enzymes is very limited but recent studies shed some light on this important issue. The human homologue of the Drosophila deubiquitination enzyme fat facets, Fam, was shown to stabilize two different substrates, the ras-target AF-6 [30] and β-catenin [31]. Moreover, Li and co-workers have recently reported that the deubiquitination enzyme USP7 can stabilize the tumor suppressor p53 through deubiquitination [32]. While each of these substrates was shown to directly interact with the relevant deubiquitinating enzyme, only for the two Fam substrates the interaction domain has been partially mapped and no attempts have been made to assess the transferability of the interacting domains to other substrates [30,31].

4. Why would substrates need stabilization signals?

A legitimate question is why proteins should carry two counteracting signals in order to regulate their proteasomal turnover when a delay of proteasomal degradation could be easily achieved by modification of the degradation signal, resulting in sub-optimal recruitment of ubiquitin ligases. One obvious answer is that the presence of two counteracting signals adds new opportunities of regulation. This is clearly illustrated by the processing of precursor proteins where the degradation signal induces proteolysis of a fragment of the protein while the remaining part escapes degradation. Hence, a processing signal should contain both a degradation and a stabilization signal. In contrast to the viral repeats that block proteasomal degradation tout-court, the nature of protein processing requires a stabilization signal that is only active once the degradation of the substrate is initiated.

An interesting possibility is that the spatial or temporal degradation of certain proteins may be regulated by conditional stabilization signals. Thus, opposite to what has been observed for many proteasome substrates, such proteins may be targeted for degradation by default and this fate can be overruled by activation of a conditional stabilization signal. An example may be the regulation of protein turnover by the levels of the specific deubiquitination enzymes. Indeed, the expression of deubiquitination enzymes changes dramatically during senescence [33] or upon oncogene activation [34], but the specific substrates of these enzymes remain to be identified. Other regulatory signals could be functional analogous to the conformational stabilization signal described by Lee and co-workers, which resists the unfoldase activity of the proteasome only after binding of its specific ligand [21]. One tantalizing observation in this respect is the regulation of the transcriptional regulator Met4 in budding yeast. The level of methionine in the culture medium controls the degradation of Met4 [35]. Yet, while addition of methionine results in ubiquitination of Met4, only in minimal medium this is followed by proteasomal degradation whereas in rich medium the ubiquitinated Met4 is stable but has an altered promotor specificity [36,37]. Although alternative explanations cannot be excluded, it is tempting to speculate that Met4 may carry a stabilization signal that is active depending on the nutrient status of the medium analogous to the ligand-dependent stabilization signals in the designed substrates.

Although ubiquitination is regarded as a major determinant of intracellular protein turnover, it has become clear that ubiquitin modification is also crucial for many other cellular processes such as membrane trafficking [38], DNA repair [39], and protein activation [40]. These different activities of ubiquitinated substrates are usually permitted by the conjugation of polyubiquitin trees via Lys residues different form the canonical Lys48, that mediates proteasomal degradation. However, the presence of strong stabilization signals could allow Lys48 polyubiquitination without proteolysis. These stable polyubiquitinated substrates may be targeted to the proteasome and mediate the recruitment of co-factors that regulate the proteolytic machinery or facilitate non-proteolytic activities of the proteasome, such as the recently discovered role of the 19S regulatory subunit in DNA repair [41].

5. Concluding remarks

While a few viral repeats clearly fulfil the criteria for functional domains with stabilizing effects, the presence of other classes of stabilization signals is still a matter of speculation. Yet, the identification and characterization of this type of regulatory sequences is a major challenge for the future. A strong focus will be on the pathogenesis of conformational diseases characterized by the accumulation of misfolded proteins that are usually rapidly destroyed by proteasomal degradation [42]. A key question is why the cells fail to clear the toxic and aggregation-prone proteins [23]. If stabilization signals are involved, overriding their effect or targeting the toxic proteins before conditional stabilization signals are activated could provide a new therapeutic approach. The immune evasion strategy of EBV based on the protective effect of the GAr illustrates another interesting opportunity. Stabilization signals may turn into powerful tools for the generation of non-immunogenic proteins or stable variants of proteasome substrate for gene therapeutic applications.

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