MINI-REVIEW

Degradation elements coincide with cofactor binding sites in a short-lived transcription factor

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Elaborate control of gene expression by transcription factors is common to all kingdoms of life. In eukaryotes, transcription factor abundance and activity are often regulated by targeted proteolysis via the ubiquitin-proteasome system (UPS). The yeast MAT α 2 (α 2) cell type regulator has long served as a model for UPS-dependent transcription factor degradation. Proteolysis of α 2 is complex: it involves at least 2 ubiquitylation pathways and multiple regions of α 2 affect its degradation. Such complexity also exists for the degradation of other UPS substrates. Here I review α 2 degradation, most notably our recent identification of 2 novel degradation elements within α 2 that overlap corepressor binding sites. I discuss possible implications of these findings and consider how principles of α 2 proteolysis may be relevant to the degradation of other UPS substrates.

ARTICLE HISTORY

Received 19 November 2015

Revised 31 January 2016

Accepted 18 February 2016

KEYWORDS

corepressor; MAT α 2 (α 2);
nucleus; proteasome;
proteolysis; transcription;
ubiquitin

Cells use multiple mechanisms to control the levels of the thousands of proteins encoded by their genes. The first step of gene expression, transcription, is often the primary mechanism for controlling the levels of a specific protein.¹ Protein levels are also frequently regulated by targeted proteolysis, which in eukaryotes is mainly accomplished by the ubiquitin-proteasome system (UPS).^{1,2} In this system, the small protein ubiquitin is covalently attached to proteins (in a process termed ubiquitylation) destined for degradation by a chambered protease known as the proteasome. Most substrates of the proteasome are modified with multiple ubiquitin molecules, usually in the form of a polyubiquitin chain. Protein ubiquitylation requires 3 enzymes: an ubiquitin-activating enzyme (E1), an ubiquitin-conjugating (Ubc) enzyme (E2), and an ubiquitin ligase (E3), that operate sequentially. Each eukaryotic genome codes for multiple E2s and many E3s, allowing a diverse range of substrates to be ubiquitylated. E3s recognize the substrate to be ubiquitylated, and are the main source of specificity in the UPS.

Among the most common substrates of the UPS are sequence-specific DNA-binding transcriptional activators and repressors—collectively called transcription factors.¹ Targeted proteolysis of transcription factors allows rapid changes to gene expression programs, which is important during development and for cellular adaptation to diverse environments. The first example of UPS-mediated transcription factor proteolysis was reported for the yeast cell-type regulator MAT α 2 (α 2).^{3,4} Subsequent studies of α 2 degradation have revealed important principles of protein degradation, some general to the UPS and others specific to the ubiquitylation pathways that target α 2.^{5,6}

Multiple degradation pathways for the yeast MAT α 2 protein

The yeast *Saccharomyces cerevisiae* can exist as 3 cell types: a non-mating diploid and 2 haploid types (a and α) that can mate to form the diploid. Most wild yeast strains are capable of rapid switching between haploid types.⁷ Because α 2 represses expression of genes for the a mating type (called a -specific genes), its rapid turnover is required for timely mating type switching from α to a cells.⁸ Efficient proteolysis of α 2 involves at least 2 distinct pathways: the Doa10 pathway and the Ubc4 pathway, named for UPS components involved (Fig. 1A).^{9–11} Perturbation of a single pathway results in only modest stabilization of α 2, whereas cells lacking both pathways show robust (though not complete) stabilization of α 2 and a concomitant block to switching from the α to a cell type.^{8,9} Many other UPS substrates, including the transcription factors p53 and Myc in mammals, are known to be targeted for degradation by multiple pathways.^{1,5} Possible reasons and implications for redundancy in UPS-dependent proteolysis have been discussed previously.⁵

The Doa10 pathway of α 2 degradation involves 2 E2 enzymes (Ubc6 and Ubc7) and the E3 Doa10, all of which associate tightly with the endoplasmic reticulum (ER)/nuclear envelope (NE).^{10,12} As expected for a transcription factor, α 2 is primarily localized to the nucleus, and Doa10 localized to the inner nuclear membrane can access nuclear proteins^{13,14}. The Doa10 pathway received the most attention in early studies of α 2 degradation, largely due to the discovery of an N-terminal fragment of α 2—called degradation signal 1, or *Deg1* (discussed in more detail below)—that allowed identification of Doa10 pathway components (Fig. 1).

Figure 1. (A) Pathways to MAT α 2 degradation. *Deg1* and *Deg2* denote degrons in α 2. (B) Degrons, degradation elements, and corepressor binding sites within MAT α 2. Numbers for each degradation element denote amino acid residues. See text for further details.

The other pathway of α 2 degradation, referred to here as the Ubc4 pathway, operates with the E2 Ubc4 and the heterodimeric E3 Slx5/Slx8 (Slx5/8).^{9,11} However, since cells lacking Slx5/8 do not show stabilization of α 2 to the extent observed with lack of Ubc4, this pathway is often named for the E2. An interesting feature of Slx5/8 is its affinity for the small ubiquitin-like modifier (SUMO) protein and its role in ubiquitylating SUMO-modified cellular proteins.¹⁵ Because of these properties, Slx5/8 and its functional homologs (including the human proteins RNF4 and RNF111) have been referred to as SUMO-targeted ubiquitin ligases (STUbLs). In addition to targeting transcription factors, STUbLs have conserved roles in genome maintenance and exhibit interesting connections to other components of the SUMO system.¹⁶ Surprisingly, despite these known properties, Slx5/8 targets α 2 in a SUMO-independent manner.¹¹ In fact, Slx5/8-dependent ubiquitylation of α 2 has been reconstituted in vitro with purified proteins that do not include SUMO.^{6,11}

Two other transcription factors—MAT α 1 and MAT α 1—regulate yeast mating and are also short-lived substrates of the UPS. MAT α 1 (α 1) functions as a transcriptional activator of α -specific genes in α -type haploid cells and, like α 2, is degraded via multiple pathways.¹⁷ One of the several E3s that target α 1 for degradation is Slx5/8; thus, α 2 and α 1 degradation may be coordinated via this pathway. MAT α 1 (α 1) is expressed in α -type haploid cells, where it is very short-lived and has no apparent function. However, it functions in a heterodimeric α 1/ α 2 complex to repress haploid-specific genes in diploid cells. Interestingly, in diploid cells α 1 and α 2 protect each other from proteolysis by direct interaction.¹⁸

Degrans and degradation elements within substrates of the UPS

Studies of UPS substrates have identified regions of these proteins important for their proteolysis.¹⁹ I will refer to any region of a protein that contributes to its degradation as a degradation

element. These regions have also been called degradation signals—abbreviated as “degrons.” The term degron is defined in different ways in the literature. Here I define a degron as a region of a protein that is both necessary and sufficient to target a protein for proteolysis.¹⁹ As such, not all degradation elements—which can theoretically be a single amino acid—meet this definition of a degron, and degrons can often be further characterized to identify degradation elements within them. As defined here, degrons are transferable. That is, the attachment of a degron (usually by genetic engineering) will confer metabolic instability to an otherwise metabolically stable protein.

A degron within a substrate of the UPS requires at least 3 properties, which can be attributed to 3 distinct degradation elements.^{19,20} The primary function of a UPS degron is to target the protein to a specific ubiquitylation machinery. Thus, degrons have an element or elements that are directly recognized by an E3 (or in some cases by an E3 accessory protein). Degron function also requires an amino acid acceptor (usually lysine) for ubiquitin attachment. Finally, a UPS degron must also contain an unstructured region that will initiate entry into the proteasome. Because UPS-dependent degradation is often spatially controlled, regions of a protein that affect its localization might also satisfy the broadest definition of a degradation element.²¹

Given the number of proteins that are known or expected substrates of the UPS and the many E3s that recognize UPS substrates—yeast express over 50 E3s and humans have as many as 500—it is not surprising that a diversity of degradation elements exist for UPS targeting.¹⁹ Elements recognized by an E3 are either part of the primary sequence of the protein, a post-translational modification (e.g., phosphorylation) to the protein, or a combination of these two.^{19,20}

Multiple degrons and degradation elements within α 2

The first natural degron to be identified, *Deg1* from α 2, is targeted by a single pathway—the Doa10 pathway (Fig. 1).^{3,10} The essentially complete stabilization observed for *Deg1*-based

Figure 2. MAT α 2 degradation is linked to its functional status. (A) Schematic for the structural organization of α 2. (B) Schematic showing α 2 bound by its cofactors at its target DNA. Cofactor binding to α 2 blocks its UPS-mediated degradation. Circles labeled “T” represent Tup1. For simplicity, this schematic shows only one subunit of the α 2 dimer bound by cofactors. The exact organization and stoichiometry of the complex is unknown. (C) Schematic depicting α 2 recognition by 2 distinct ubiquitylation pathways. INM = inner nuclear membrane. Hypothetical factors and steps in α 2 recognition are marked with a (?) symbol. See text for further details.

current in vitro assay, I hypothesize that the in vitro reaction would be much more efficient if the hypothetical Slx5/8 cofactor were present. Based on the hydrophobicity of the linker element, I have proposed that the linker binds a chaperone protein that functions with Slx5/8 (see below).⁶

Other transcription factors with multiple degrons

Like $\alpha 2$, other transcription factors are known or hypothesized to have multiple degrons.^{1,17} MAT α 1 contains at least 2 degrons, one that is targeted by Slx5/8 and one that is targeted by a different ligase or ligases.¹⁷ The Rpn4 transcriptional activator also has at least 2 degrons.²⁹ Interestingly, one of the degrons in Rpn4 is degraded by the proteasome in an ubiquitin-independent manner, whereas the other degron is clearly ubiquitin-dependent. A few other proteins, all of which have a region of notable intrinsic disorder, are thought to be degraded by the proteasome in an ubiquitin-independent manner.²⁹ As previously mentioned, the need for an unstructured region, usually at protein termini, has been suggested for all proteasome substrates—even those targeted to the proteasome by ubiquitin.² Thus, studies that employ mutagenesis of short-lived proteins should consider potential effects on proteasomal degradation in addition to effects on ubiquitylation.

The role of corepressors in $\alpha 2$ degradation

The two Ubc4 pathway degradation elements recently identified within $\alpha 2$ overlap binding sites for the $\alpha 2$ corepressors Mcm1 and Ssn6 (Figs. 1 and 2).⁶ The hydrophobic residues within the linker element make intimate contact with Mcm1, as observed in a crystal structure of a ternary complex of $\alpha 2$, Mcm1, and the target operator DNA.³⁰ While these hydrophobic residues are part of a β -strand when bound to Mcm1, it remains unclear how they are structured when not bound to Mcm1. One possibility is that these residues bind another protein—perhaps a chaperone—in the absence of Mcm1. In such a situation, the structure of the linker region may not resemble that observed in the crystal structure of the ternary complex. We recently showed that $\alpha 2$ is still rapidly degraded in the absence of Mcm1.⁶ In fact, $\alpha 2$ degradation is slightly faster in *mcm1* Δ yeast, which I hypothesize is due to enhanced exposure of the degradation element in the $\alpha 2$ linker.

A precedent for corepressors blocking the exposure of degradation elements was provided in a previous study of $\alpha 2$ degradation.³¹ A screen for proteins that when overexpressed stabilize *Deg1* fusions yielded the Tup1 protein. Tup1 overexpression also modestly stabilized full-length $\alpha 2$. Furthermore, co-overexpression of both Tup1 and Ssn6 led to robust stabilization of $\alpha 2$. Tup1 and Ssn6 form a corepressor complex that functions with multiple sequence-specific transcription factors.³² Tup1 interacts with the N-terminus of $\alpha 2$, consistent with its ability to block access to *Deg1* (Fig. 2B). Ssn6 interacts with the homeodomain of $\alpha 2$ —in a region recognized by Slx5/8.^{6,33} Thus, we have proposed a model in which Ssn6 binds to the $\alpha 2$ homeodomain and blocks access by Slx5/8.⁶ Evidence for a direct competition between Ssn6 and Slx5/8 is lacking, but experiments using the aforementioned in vitro assay with a

purified functional fragment of Ssn6 could be attempted in the future.

Developing paradigms for transcription factor degradation

It would be interesting to determine whether the degradation of other transcriptional repressors is affected by their interaction with corepressors, particularly the general corepressors Tup1 and Ssn6. Other short-lived proteins that function with Tup1 and Ssn6 include the repressors Mig1 and Cup9.^{34,35} Cup9 degradation is mediated by the Ubr1 ubiquitin ligase, and its role in peptide import has been studied extensively.^{35–39} Though Ubr1 is best known for its role in degradation of N-end rule substrates, Ubr1 targeting of Cup9 does not depend on the N-terminus of Cup9 but rather a region near its C-terminus.³⁶ How Tup1 and Ssn6 interact with Cup9 remains to be determined.

For both Cup9 and $\alpha 2$, their enhanced stability in UPS mutants leads to repression in circumstances when transcriptional repression should be relieved.^{8,35} Thus, UPS-mediated turnover of these repressors acts to limit their abundance, allowing rapid changes to the transcriptional programs that they control. This relatively straightforward role for UPS-mediated proteolysis contrasts with a situation observed for many transcriptional activators. For these activators, their UPS-mediated proteolysis actually promotes the transcriptional program under their positive control.^{1,40} While this counterintuitive phenomenon is not fully understood (as discussed extensively by Geng et al.),¹ it is clear that for many transcriptional activators, their degrons closely or completely overlap their transcriptional activation domains.¹ In a model that involves phosphorylation of activators, Geng et al. propose that UPS-mediated proteolysis clears spent (no longer active) activators from chromatin, allowing a fresh activator to take its place. Geng et al. also note that a separate mode of activator degradation, when they are not bound to their specific gene sites, plays the more straightforward role of limiting the abundance of the transcription factor. Although UPS-mediated proteolysis has not been observed to enhance repression, this mechanism could theoretically exist for repressors. Nevertheless, the fact that corepressors affect the rate of $\alpha 2$ degradation suggests that cells can link UPS-mediated proteolysis to the functional status of a transcription factor whether it be a repressor or activator.

Hydrophobicity and molecular chaperones in $\alpha 2$ degradation

In addition to corepressors, other proteins that bind $\alpha 2$ may impact its degradation. Given its 2 regions of exposed hydrophobicity—the amphipathic helix in *Deg1* and the consecutive hydrophobic amino acids in the linker, both of which are degradation elements—one might expect that $\alpha 2$ would bind chaperone proteins (at least temporarily). Chaperones of the Hsp70 family and their co-chaperones of the Hsp40 family are known to prevent the aggregation of proteins with exposed hydrophobicity—the main characteristic of misfolded proteins.²⁶ These same chaperone families play important roles in several ubiquitylation pathways, as reviewed by Shiber and Ravid.²⁶

For $\alpha 2$ degradation, perhaps the most interesting question is whether chaperones are involved in substrate recognition by Doa10 and/or Slx5/8 (Fig. 2C). While potential roles for chaperones in the degradation of full-length $\alpha 2$ have not been investigated, one study provided evidence for the involvement of the Hsp40 chaperone Sis1 in the degradation of a membrane-embedded *Deg1*-fusion.²⁸ There is additional evidence for Hsp70/Hsp40 chaperone involvement in the degradation of other Doa10 substrates.^{12,24,27,28,41} As previously mentioned, direct interaction between Doa10 and *Deg1* (or $\alpha 2$) has never been demonstrated. Presumably this interaction is transient and ubiquitylation need not be processive—that is, Doa10 might release its substrates between ubiquitin additions. However, there may be mechanisms to keep Doa10 substrates close to the Doa10 machinery on the ER/NE and such mechanisms may be lost after cells are lysed and/or membranes are solubilized in detergent. For proteins bearing the artificial Doa10-dependent degron CL1, the chaperones Ydj1 (Hsp40) and Ssa1 (Hsp70) are proposed to be part of a network of proteins that create a platform for ubiquitylation and degradation on the cytosolic face of the ER membrane.^{12,27} A subpopulation of Ydj1 is known to acquire a lipidic tail that anchors it in the ER/NE membrane. Another Hsp40 protein, Hlj1, is integral to the ER membrane and has also been implicated in degradation of proteins at the ER, including Doa10 substrates.^{41,42}

For the Ubc4 pathway of $\alpha 2$ degradation, one hypothesis is that a chaperone protein might bind both the $\alpha 2$ linker and Slx5/8 to accelerate the Slx5/8-mediated ubiquitylation of $\alpha 2$ (Fig. 2C). This idea can theoretically be tested using the in vitro assay described earlier, with either cell extracts as a potential source of the appropriate chaperone(s) or with purified candidate chaperones. While the ability of chaperones to modulate Slx5/8 activity has never been demonstrated biochemically, Slx5/8 has been genetically linked to one chaperone, the Hsp40-family chaperone Api1.⁴³

Theoretically, a chaperone that operates with both Doa10 and Slx5/8 could have a large effect on $\alpha 2$ degradation. However, given that yeast—like all eukaryotes—express many chaperones that are functionally redundant,^{26,43} it is unlikely that a chaperone protein encoded by a single gene would be uniquely responsible for such a function. For this and other reasons, biochemical studies of chaperone function in $\alpha 2$ degradation may be more enlightening than genetic approaches.

Concluding remarks

In-depth analyses of cellular processes using tractable model systems have continually produced discoveries fundamental to our understanding of life that are often relevant to human health and disease. The use of yeast to study proteolysis by the UPS is one of the many great examples of the power of basic biomedical science. The $\alpha 2$ repressor was the first endogenous substrate of the UPS to be characterized and continues to be an important model UPS substrate.

Many questions remain about the UPS and, in particular, transcription factor degradation. Are there cases in which transcriptional repressor degradation enhances repression, similar to what has been observed for activators? Do transcriptional corepressors affect repressor degradation in cases other than

$\alpha 2$? With regard to chaperones, what is their exact role in $\alpha 2$ degradation and are these roles more general to the UPS?

A long-term goal of the UPS field is the ability to predict whether a protein is short-lived, and if so, which pathway(s) target it for proteolysis. This is no trivial task. Cells express many E3s—the bulk of which remain uncharacterized—and each E3 has, or is expected to have, multiple substrates. In addition, whether a protein is degraded can be influenced by its subcellular localization, its interaction partners, post-translational modifications to the protein (besides ubiquitin), and the chemical properties of the intracellular environment. Future studies of transcription factor proteolysis are expected to provide further insight into these issues.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

Acknowledgments

I thank Mark Hochstrasser (Yale University) for continued support and feedback on the manuscript. I also thank Jason Berk (Yale University) for critical reading of the manuscript.

Funding

My work is supported by NIH grants GM046904 and GM053756 (to M.H.) and previously by a Ruth L. Kirschstein National Research Service Award (F32GM097794).

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