The F-Box Protein, Ufo1, Maintains Genome Stability by Recruiting the Yeast Mating Switch Endonuclease, Ho, for Rapid Proteasome Degradation

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Key words: Ho, Ddi1, proteasome, DNA damage response, nuclear export

Abstract

We describe a unique E3, the F-box protein, Ufo1, of yeast. Ufo1 recruits the mating switch endonuclease, Ho, to the SCF complex for ubiquitylation. In addition to the F-box and WD40 protein-protein interaction domains found in all F-box proteins, Ufo1 has a unique domain comprising multiple copies of the ubiquitin-interacting motif. Ufo1 interacts with the UbL-UbA protein, Ddi1, via its UIMs, and this is required for turnover of SCF^{Ufo1} complexes. This is a novel function for an UbL-UbA protein. Deletion of the genomic *UFO1* UIMs is lethal and our data indicate that Ufo1ΔUIM acts as a dominant negative leading to inhibition of the SCF pathway of substrate degradation and to cell cycle arrest. Furthermore, we found that Ddi1 is required for the final stages of degradation of Ho endonuclease. In the absence of Ddi1, Ho does not form a complex with the 19S RP and is stabilized. Stabilization of Ho leads to perturbation of the cell cycle and to the formation of multi-budded cells. Our experiments uncover a novel role for the ubiquitin-proteasome system in maintenance of genome stability.

IMAJ 2006;8:246–248

The SCF E3 complex mediates G1/S cell cycle progression

Cell cycle progression is driven by two ubiquitin ligase (E3) complexes that ubiquitylate specific substrates at defined stages of the cell cycle for degradation in the proteasomes. The SCF (Skp1/Cdc53/F-box protein) is active mainly at G_1/S and is responsible for ubiquitylation of G_1 cyclins, and of the cyclin-dependent kinase inhibitor, yeast Sic1/human p27, among others. The SCF scaffold, yeast Cdc53/human Cullin, binds at its N-terminus a substrate-recruiting module comprising the Skp1 adaptor and an F-box protein [1].

Multiple F-box proteins each recognize a subset of degradation substrates; these are usually marked by phosphorylation, thus linking proteasomal degradation with the regulatory protein kinase signal transduction cascades [1,2]. At its C-terminus the SCF scaffold has a catalytic module comprising the RING protein, Rbx1, which serves as a landing pad for ubiquitin-charged E2, in yeast, Cdc34. Most F-box proteins have an F-box domain that binds Skp1, and a WD40 or LRR protein-protein interaction domain to which the degradation substrate binds. Subunits of the SCF co-purify with proteasomes [3]; however, extraction of ubiquitylated substrates from the SCF complex and their transfer to the proteasome is not completely understood. Most F-box proteins are short-lived proteins and are ubiquitylated within the SCF complex and degraded; however, recent reports indicate that the second cell cycle E3 complex, the APC, may also play a role in their turnover [4].

UIM = ubiquitin-interacting motif
SCF = Skp1/Cdc53/F-box protein

The mating switch endonuclease, Ho, is regulated by rapid degradation

In the course of a study on the yeast mating switch endonuclease, Ho, we identified a novel F-box protein, Ufo1, that recruits Ho to the SCF [5,6]. Ufo1 is unique among F-box proteins in that its transcription is induced in response to DNA damage [7]. Ho is very efficiently imported into the nucleus [8] and makes a site-specific double-strand break at the mating type locus, *MAT*, in late G_1. The double-strand break is repaired by gene conversion using as a template a copy of *MAT* sequence found at one of the silent mating-type cassettes. This regenerates the Ho cognate site. We found that in addition to tight transcriptional regulation of *HO* [9], the protein is rapidly degraded via the ubiquitin-26S proteasome system with a half-life of about 8 minutes [5]. Ho is marked for degradation by functions of the DNA damage response, specifically a pathway defined by *MEC1*, *RAD9* and *CHK1* [5]. This DNA damage response sub-pathway leads to phosphorylation of Ho on residue Thr225 and this is essential for nuclear export of Ho. Mutation of Thr225 to alanine, or deletion of Mec1, results in nuclear accumulation of Ho that is not degraded. Ho nuclear export occurs via the nuclear exportin, Msn5, and in *Δmsn5* cells Ho trapped in the nucleus is stabilized [Figure 1]. Moreover, *HO* expressed from its native promoter and stabilized by deletion of Msn5 leads to genome instability with accumulation of multi-budded cells in which both daughter nuclei are often retained in the mother cell. Our interpretation is that constant Ho activity perturbs the synchronization between the DNA synthesis, and the bud emergence and nuclear migration pathways of the cell cycle.

The novel F-box protein, Ufo1

Ufo1 resembles the human Cdc4/Fbw7 F-box protein; however, it has a unique C-terminal domain comprising multiple copies of the ubiquitin-interacting motif. This motif is predicted to bind ubiquitin and ubiquitin-domain proteins [10]. Deletion of the UIMs of genomic UFO1 is lethal; ectopic expression of

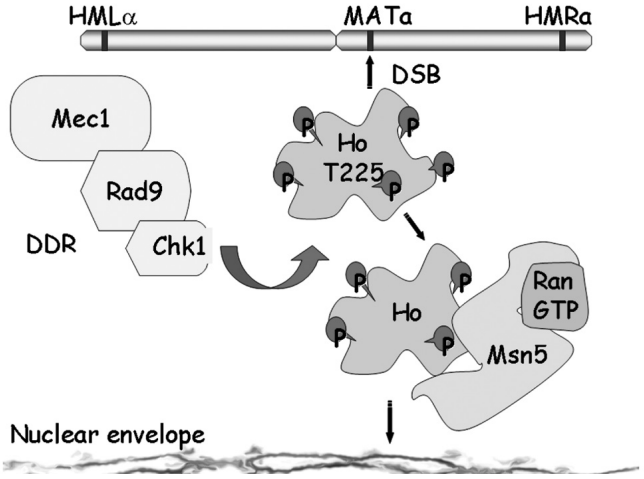

Figure 1. Diagram illustrating phosphorylation of Ho by functions of the DNA damage response (DDR) within the nucleus. Phosphorylation of HoT225 is essential for nuclear export of Ho that is mediated by the karyopherin, Msn5 [6].

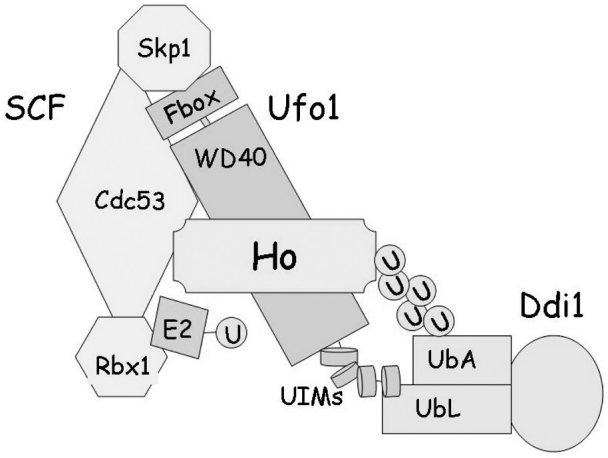

Figure 2. Diagram showing the interactions between Ufo1 and the SCF subunits, Skp1 and Cdc53 [8]; between ubiquitylated Ho and the UbA domain of Ddi1 [18]; and between the UIMs of Ufo1 and the UbL domain of Ddi1 [11]. We hypothesize that these interactions may take place in a complex with the 19S RP of the proteasome.

truncated UFO1 without UIMs leads to cell cycle arrest. Deletion of the UIMs leads to stabilization of Ufo1, which may inhibit turnover of $SCF^{Ufo1}\Delta^{UIM}$ complexes. Cells in which UFO1ΔUIM is over-expressed arrest with long buds and resemble *skp*1 mutants at the restrictive temperature, indicative of lack of SCF activity. Therefore, we contend that stabilized Ufo1ΔUIM acts as a dominant negative and inhibits the SCF pathway of substrate degradation. This is supported by experiments showing that substrates of other F-box proteins, e.g., Cln2, recruited to the SCF by Grr1, and Sic1, a ubiquitylation substrate of the F-box protein, Cdc4, accumulate in cells in which UFO1ΔUIM is over-expressed. Ho degradation is not affected by deletion of the Ufo1 UIMs, indicating that this domain is not required for Ufo1-mediated substrate ubiquitylation [11].

Ufo1 binds the UbL-UbA protein, Ddi1, via its UIMs

The UbL-UbA proteins, exemplified by Rad23, Dsk2, and Ddi1, are proposed to act as adaptors and to deliver ubiquitin-conjugated substrates to the proteasome [12,13]. The UbA domain [14] shows a high affinity for K48-linked polyubiquitin chains [13,15], whereas the N-terminal ubiquitin-like domain adopts a ubiquitin fold and binds the 19S RP [16,17]. We found indeed that the Ufo1 UIMs bind all three UbL-UbA proteins; however, full-length Ufo1 shows specificity for the UbL-UbA protein, Ddi1 (DNA damage inducible) [11].

Ufo1 is an unusually stable F-box protein and is further stabilized in Δddi1 cells

Given the interaction of Ufo1 with Ddi1 and the stabilization of Ufo1 by deletion of its UIMs we tested whether the half-life of full-length Ufo1 would be extended in Δ*ddi*1 cells. Indeed we found a retardation of Ufo1 turnover compared with wild-type cells, or in *rad*23 or *dsk*2 mutants. Furthermore, in Δ*ddi*1 cells full-length Ufo1 could lead to cell cycle arrest and the appearance of cells with long buds [11].

Ddi1 is required for the final stages of Ho degradation

Ho interacts specifically with Ddi1 – and this only when produced in cells that support its ubiquitylation. Ddi1 interacts with ubiquitylated Ho via its UbA domain and with the 19S RP via its UbL domain. Both domains of Ddi1 are required for complex formation between Ho and the 19S [Figure 2]. In Δ*ddi*1 mutants, stabilized Ho accumulates in the cytoplasm [18].

Conclusions

The UbL-UbA protein, Ddi1, interacts both with ubiquitylated Ho endonuclease and with the F-box protein, Ufo1. This suggests that it functions both in substrate extraction and in SCF^{Ufo1} complex disassembly. Given the interaction between Ddi1 and the 19S RP these processes may occur in a megacomplex comprising SCF and proteasome. All the functions involved in rapid elimination of Ho endonuclease are involved in the cellular response to DNA damage. These include Mec1, Rad9, Chk1, Ufo1, and Ddi1. Thus, elucidation of the pathway of Ho degradation has revealed a unique role for the ubiquitin-26S proteasome system in the maintenance of genome stability.

UbL = ubiquitin-like

Acknowledgments. This research was supported by the Israel Cancer Association (ICA), the Israel Cancer Research Fund (ICRF), the Association for International Cancer Research (AICR), the German-Israel Research Fund (GIF), and by FP5 EU contract HPRN-CT-2002-00238. L. Kaplun is a post-doctoral fellow supported by the GIF. A. Bakhrat is a Kreitman Fellow of the Ben-Gurion University Graduate School. We thank Emilia Klyman for technical assistance.

References

1. Skowyra D, Koepp DM, Kamura T, et al. Reconstitution of G1 cyclin ubiquitination with complexes containing SCFGrr1 and Rbx1. *Science* 1999;284:662–5.
2. Skowyra D, Craig KL, Tyers M, Elledge SJ, Harper JW. F-box proteins are receptors that recruit phosphorylated substrates to the SCF ubiquitin-ligase complex. *Cell* 1997;91:209–19.
3. Verma R, Chen S, Feldman R, et al. Proteasomal proteomics: identification of nucleotide-sensitive proteasome-interacting proteins by mass spectrometric analysis of affinity-purified proteasomes. *Mol Biol Cell* 2000;11:3425–39.
4. Vodermaier HC. APC/C and SCF: controlling each other and the cell cycle. *Curr Biol* 2004;14:R787–96.
5. Kaplun L, Ivantsiv Y, Kornitzer D, Raveh D. Functions of the DNA damage response pathway target Ho endonuclease of yeast for degradation via the ubiquitin-26S proteasome system. *Proc Natl Acad Sci* USA 2000;97:10077–82.
6. Kaplun L, Ivantsiv Y, Bakhrat A, Raveh D. DNA damage response-mediated degradation of Ho endonuclease via the ubiquitin system involves its nuclear export. *J Biol Chem* 2003;278:48727–34.
7. Jelinsky SA, Samson LD. Global response of Saccharomyces cerevisiae to an alkylating agent. *Proc Natl Acad Sci* USA 1999;96:1486–91.
8. Bakhrat A, Baranes K, Krichevsky O, Pietrokovski S, Raveh D. *J Biol Chem* 2006; E-pub doi:10.1074/jbc.M600238200.
9. Cosma MP, Tanaka T, Nasmyth K. Ordered recruitment of transcription and chromatin remodeling factors to a cell cycle and developmentally regulated promoter. *Cell* 1999;97:299–311.
10. Hofmann K, Falquet L. A ubiquitin-interacting motif conserved in components of the proteasomal and lysosomal protein degradation systems. *Trends Biochem Sci* 200126:347–50.
11. Ivantsiv Y, Kaplun L, Tzirkin-Goldin R, Shabek N, Raveh D. Turnover of SCF^{Ufo1} complexes requires the UbL-UbA motif protein, Ddi1. *Mol Cell Biol* 2006;26: in press.
12. Hartmann-Petersen R, Hendil KB, Gordon C. Ubiquitin binding proteins protect ubiquitin conjugates from disassembly. FEBS *Lett* 2003;535:77–81.
13. Wilkinson CR, Seeger M, Hartmann-Petersen R, et al. Proteins containing the UBA domain are able to bind to multi-ubiquitin chains. *Nat Cell Biol* 2001;3:939–43.
14. Hofmann K, Bucher P. The UBA domain: a sequence motif present in multiple enzyme classes of the ubiquitination pathway. *Trends Biochem Sci* 1996;21:172–3.
15. Raasi S, Orlov I, Fleming KG, Pickart CM. Binding of polyubiquitin chains to ubiquitin-associated (UBA) domains of HHR23A. *J Mol Biol* 2004;341:1367–79.
16. Elsasser SR, Gali R, Schwickart M, et al. Proteasome subunit Rpn1 binds ubiquitin-like protein domains. *Nat Cell Biol* 2002;4:725–30.
17. Walters KJ, Kleijnen MF, Goh AM, Wagner G, Howley PM. Structural studies of the interaction between ubiquitin family proteins and proteasome subunit S5a. *Biochemistry* 2002;41:1767–77.
18. Kaplun L, Tzirkin R, Bakhrat A, Shabek N, Ivantsiv Y, Raveh D. The DNA damage-inducible UbL-UbA protein Ddi1 participates in Mec1mediated degradation of Ho endonuclease. *Mol Cell Biol* 2005;25:5355–62.

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