

Allosteric Activation of the Ubiquitin Ligase UBR1 by Short Peptides:  
Molecular Mechanisms and Physiological Functions

Thesis by  
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In Partial Fulfillment of the Requirements  
for the Degree of  
Doctor of Philosophy

California Institute of Technology  
Pasadena, California  
2002

(Defended November 21, 2001)

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## ACKNOWLEDGMENTS

Despite bumpy roads at the beginning of my journey, I have had a wonderful time learning to ask the right questions and design neat experiments in Alex's lab; the thrilling moments of coming up with good ideas are unforgettable.

I am very grateful to Alex for his patience to allow me to grow at my own pace, for the freedom to work independently in his lab, and for driving me to be my best. It has been a great experience to observe closely his creativity, rigor, and above all, his devotion to science. I am thankful for his time and efforts in editing my thesis. I also want to thank my thesis committee— Ray Deshaies, Bill Dunphy, Judith Campbell and Giuseppe Attardi for their advice and encouragement over the years.

I particularly thank Glenn C. Turner, my collaborator and a good personal friend, for his good ideas, in-depth discussions and excellent editorial efforts. His "refuse-to-feel-depressed" attitude has been inspiring to me over the years. In addition, Ailsa Webster helped me get started with protein purification, and her encouragement kept me going for a long way in the early days. Larry Peck showed me how to be meticulous with my experiments, and his patience in correcting my everyday English benefited me greatly. I thank Xiao-Dong Su for his abundant help, he made me understand what "a friend in need is a friend indeed" is all about.

My sincere thanks also go to Drs. Tau-Mu Yi and Scott Stevens for their numerous suggestions and reagents, past and current Varshavsky lab members: Marty Gonzalaz, Hai Rao, Takafumi Tasaki, Yong Tae Kwon, Ilia Davydov, Chris

Byrd, Jun Sheng, Youming Xie, Zan-Xian Xia, Rong-Gui (Cory) Hu – thanks for strains, plasmids, cell lines and many happy hours in the lab.

Finally, I thank my wife Irene Ping Ren and my parents in China, Shi-Wei Du and Chui-Zhu Xu, for their unconditional love and support, and for their faith in me, in good times and bad times.

## ABSTRACT

The N-end rule relates the *in vivo* half life of a protein to the identity of its N-terminal residue. UBR1, the E3 of the N-end rule pathway in *Saccharomyces cerevisiae*, targets proteins that bear destabilizing N-terminal residues for Ub-dependent, processive degradation. UBR1 binds protein substrates or dipeptides through two distinct sites: the type 1 site, specific for basic residues, and the type 2 site, specific for bulky hydrophobic residues. UBR1 also recognizes an internal degradation signal of the 35 kDa homeodomain protein CUP9, a transcriptional repressor of the di- and tripeptide transporter PTR2.

Here I report that the internal degradation signal of CUP9 is recognized by UBR1 through its third, distinct substrate-binding site. Occupation of the type 1 or type 2 sites of UBR1 by dipeptides allosterically stimulates the UBR1-dependent multi-ubiquitylation of CUP9 in an *in vitro* system, which consists of purified components of the yeast N-end rule pathway. UBR1 is the first E3 shown to be allosterically regulated by small compounds. This regulation underlies, *in vivo*, the accelerated UBR1-dependent degradation of CUP9 in the presence of dipeptides with destabilizing N-terminal residues. The result is a positive feedback circuit that controls the peptide import in *S. cerevisiae*. Specifically, the imported dipeptides bind to UBR1 and accelerate the UBR1-dependent degradation of CUP9, thereby derepressing the transcription of *PTR2* and increasing the cell's capacity to import peptides.

I also describe a new, autoinhibition-based molecular mechanism underlying the activation of UBR1 by dipeptides. UBR1 is an autoinhibited protein, in that the binding of dipeptides to the type 1 and type 2 sites of UBR1 enhances the dissociation of the C-terminal autoinhibitory domain of UBR1 from

its substrate-binding N-terminal region. Moreover, this dissociation, which allows the interaction between UBR1 and CUP9, is strongly increased only if both type 1 and type 2 sites of UBR1 are occupied by dipeptides. An autoinhibitory mechanism discovered in the *S. cerevisiae* UBR1 is likely to recur in metazoan homologs of UBR1, and may also be involved in controlling the activity of other Ub-dependent pathways.

## TABLE OF CONTENTS

<b>Acknowledgments</b>	iii
<b>Abstract</b>	v
<b>Table of Contents</b>	vii
<b>Chapter 1</b>	
The ubiquitin system and the N-end rule pathway	1
Introduction	2
The Ub/proteasome system in <i>S. cerevisiae</i>	4
The N-end rule pathway	15
N-degron and its applications	18
Components of the N-end rule pathway	23
Physiological substrates and functions of the N-end rule pathway	39
Future directions	48
References	55
<b>Chapter 2</b>	
Establishment of an <i>in vitro</i> ubiquitylation system with purified components of the <i>S. cerevisiae</i> N-end rule pathway	71
Introduction	72
Materials and methods	73
Results and discussion	80
References	88
Figures and legends	91

**Chapter 3**

Peptides accelerate their uptake by activating a ubiquitin-dependent proteolytic pathway	96
Abstract	97
Introduction, results and discussion	97
Methods	104
References	107
Figures and legends	111

**Chapter 4**

Pairs of dipeptides synergistically activate the binding of substrate by ubiquitin ligase through dissociation of its autoinhibitory domain	119
Abstract	120
Introduction, results and discussion	120
References and notes	132
Figures and legends	137

**Chapter 5**

Missions unfinished-Experiments related to the N-end rule pathway	149
5.1 The internal degron of CUP9	150
5.2 Interaction of the type 1 and type 2 sites of UBR1 with dipeptides or other derivatives of amino acids	157
5.3 Cofractionation of Leu-ILV1 <sup>34-576</sup> and TDH2/TDH3 with <sup>f</sup> UBR1 <sup>h</sup>	164
5.4 Purification of the recombinant <i>S. cerevisiae</i> NTA1 and its enzymatic activity assay	170

5.5 Multicopy suppressors of ATE1 overexpression-mediated  
growth arrest of *S. cerevisiae* cells

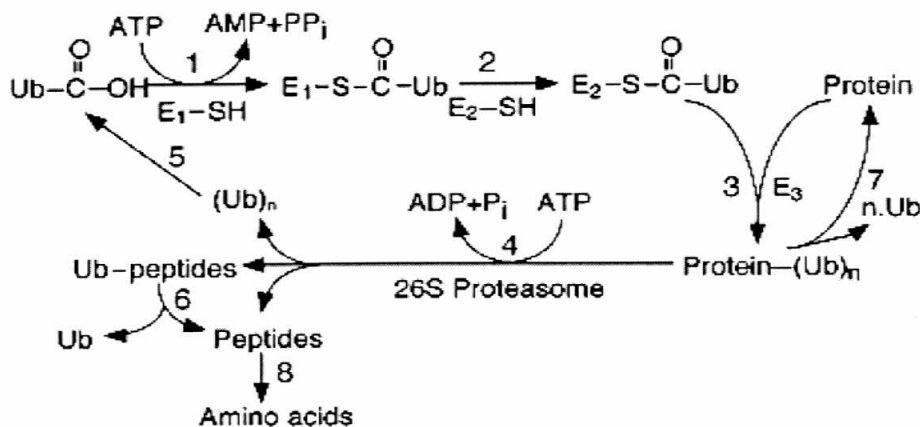
## Chapter 1

### The ubiquitin system and the N-end rule pathway

## Introduction

Intracellular protein homeostasis involves both protein synthesis and degradation. Protein degradation eliminates damaged or misfolded proteins and controls the concentrations of many regulatory proteins. In eukaryotes, the ATP-dependent ubiquitin (Ub)/proteasome system is responsible for the bulk of selective protein degradation [1, 2]. As a result, the Ub system is essential for the control of many cellular processes, including cell cycle progression, transcriptional regulation, oxygen sensing and stress responses.

Ub is an abundant 76-residue protein that is highly conserved in eukaryotes. The essentially unchanged amino acid sequence and spatial structure of Ub from fungi to humans underscore its functional importance. In early 1980s, through biochemical dissection of the ATP-dependent proteolytic system in rabbit reticulocyte lysate, Hershko and co-workers [3-5] [6] discovered that the heat-stable Ub (initially named APF-1) was an essential component of the ATP-dependent protein degradation system. They elucidated the conserved enzymatic reactions that Ub is involved (Figure 1.1):



**Figure 1.1** Enzymatic reactions of the ubiquitin system (See text for details) [2]

Ub is first activated by ubiquitin-activating enzyme (E1), with the C-terminal glycine of Ub forming, in an ATP-dependent reaction, a high energy thioester bond with a conserved Cys residue of E1. The activated Ub is then transferred to one of the ubiquitin-conjugating enzymes (E2) through the transesterification reaction. An E2 enzyme usually forms a complex with a specific ubiquitin ligase (E3) which recognizes the degradation signal (degron) of a protein substrate. Through coordinated actions of E2 and E3, the carboxyl group of the C-terminal Gly residue of Ub forms an isopeptide bond with the  $\epsilon$ -amino group of a lysine residue of a protein substrate. In most cases, after the conjugation of the first Ub moiety, a substrate-linked multi-Ub chain is built processively on the substrate. In a multi-Ub chain, Ub moieties are linked through isopeptide bond between the C-terminal Gly of one Ub and the Lys48 of an adjacent Ub [7]. In at least one Ub-dependent pathway, an additional distinct component, termed E4, is required for efficient Ub chain elongation [8]. The multiubiquitylated substrate is recognized, unfolded and finally degraded to short peptides through the action of a ~2,000 kDa, ATP-dependent multicatalytic protease termed 26S proteasome. It consists of a 20S core particle and two 19S regulatory particles that cap both ends of a 20S core particle [9, 10].

Physiological functions of the Ub-dependent proteolysis were left unaddressed by the early work with the cell-free reticulocyte lysate system. In 1984, Varshavsky and co-workers discovered that the temperature-dependent cell cycle arrest of the mouse cell line ts85 is caused by a temperature-sensitive E1 [11, 12]. These results provided the first evidence that the Ub system is essential for the cell-cycle progression. They further discovered that Ub is required for the bulk of selective protein degradation in living cells [11, 12].

One of the most striking features of Ub system is its exquisite selectivity. Thus, some of the fundamental questions are: What constitutes a degron? How is it recognized? How are the recognition and subsequent degradation regulated? What are specific functions of the Ub-mediated proteolysis of a specific protein substrate? My work, described in this thesis, has produced several direct answers to the above questions in one pathway of the Ub system — the N-end rule pathway in the yeast *S. cerevisiae*.

### **The Ub/proteasome system in *S. cerevisiae***

Molecular genetic analysis of the physiological functions of the Ub/proteasome system, using *S. cerevisiae* as a model organism, was pioneered by Varshavsky's lab in mid-1980s. Their groundbreaking research laid foundations for subsequent development of the entire Ub field [13]. The yeast *S. cerevisiae* continues to be a favorite model organism, because of the powerful genetic tools that are available with this fungus, and because its relatively small genome has been fully sequenced. I will briefly review our current understanding of the components of the Ub/proteasome system in *S. cerevisiae* (Figure 1.2).

### Ub genes

Ub is expressed in *S. cerevisiae* as fusion proteins encoded by four genes: *UBI1*, *UBI2*, *UBI3* and *UBI4* [14, 15]. *UBI1* and *UBI2* encode identical proteins. These Ub fusion proteins are efficiently (cotranslationally) cleaved *in vivo* to yield Ub and "tail" proteins through the action of Ub-specific processing proteases (UBPs). The "tails" of *UBI1*, *UBI2* and *UBI3* are ribosomal proteins, which are

