191 Tooze, S. A., and Huttner, W. B., Cell-free protein sorting to the regulated and constitutive secretory pathways. Cell *60* (1990) 837–847.
192 Von Zastrow, M., Castle, A. M., and Castle, J. D., Ammonium chloride alters secretory protein sorting within the maturing exocrine storage compartment. J. biol. Chem. *264* (1989) 6566–6571.
193 Warren, L., Stimulation of lysosomal enzyme secretion by growth factors. Exp. Cell Res. *190* (1990) 133–136.
194 Wilde, C. J., Addey, C. V. P., and Knight, C. H., Regulation of intracellular casein degradation by secreted milk proteins. Biochim. biophys. Acta *992* (1989) 315–319.
195 Winkler, J. R., and Segal, H. L., Swainsonine inhibits glycoprotein degradation by isolated rat liver lysosomes. J. biol. Chem. *259* (1984) 15369–15372.
196 Woods, J. W., Goodhouse, J., and Farquhar, M. G., Transferrin receptors and cation-independent mannose-6-phosphate receptors deliver their ligands to two distinct subpopulations of multivesicular endosomes. Eur. J. Cell Biol. *50* (1989) 132–143.
197 YaDeau, J. T., and Blobel, G., Solubilization and characterization of yeast signal peptidase. J. biol. Chem. *264* (1989) 2928–2934.
198 Zammit, V. A., and Easom, R. A., Regulation of hepatic HMG-CoA reductase in vivo by reversible phosphorylation. Biochim. biophys. Acta *927* (1987) 223–228.

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Genetic analysis of ubiquitin-dependent protein degradation

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Abstract. Selective degradation of cellular proteins serves to eliminate abnormal proteins and to mediate the turnover of certain short-lived proteins, many of which have regulatory functions. In eukaryotes a major pathway for selective protein degradation is ATP-dependent and is mediated by the ubiquitin system. This pathway involves substrate recognition by components of a ubiquitin-protein ligase system, covalent attachment of ubiquitin moieties to proteolytic substrates, and subsequent degradation of these conjugates by a multicatalytic protease complex. Recent genetic evidence suggests that the remarkable selectivity of this process is largely controlled at the level of substrate recognition by the ubiquitin ligase system. In *Saccharomyces cerevisiae*, ubiquitin-conjugating enzymes UBC1, UBC4 and UBC5 have been identified as key components of this highly conserved degradation pathway. Genetic analysis indicates that ubiquitin-dependent proteolysis is essential for cell viability and that UBC4 and UBC5 enzymes are essential components of the eukaryotic stress response.
Key words. Yeast; protein degradation; ubiquitin conjugating enzymes; signals for proteolysis; stress response.

Introduction

Protein levels in living cells are controlled both by synthesis and degradation. In every cell, proteins with relatively long half-lives coexist with proteins with short half-lives. This remarkable difference in protein stability requires mechanisms which precisely control the selectivity of the degradation process. Selective degradation is involved both in the turnover of short-lived proteins such as crucial cellular regulators, and in the selective elimination of abnormal, mislocalized and misassembled proteins.

Eukaryotes have evolved different degradative pathways. Protein degradation via lysosomes is strongly induced under starvation conditions and appears to be rather unselective. However, recent evidence suggests that a specific sequence motif (e.g. Lys-Phe-Glu-Arg-Gln) [7] may target some long-lived proteins for lysosomal degradation. A member of the hsp70 stress protein family was found to bind a protein bearing this sequence motif, and may function in the translocation of proteins across lysosomal membranes. Recently, a novel degradation pathway located in the endoplasmic reticulum has been described [21]. This pathway appears to be responsible for the elimination of newly-synthesized membrane proteins passing through the secretory pathway when these have failed to fold correctly or to assemble into the requisite oligomeric complexes.

A major pathway for the selective degradation of abnormal and short-lived proteins in the cytosol depends both on ATP and ubiquitin [24]. Biochemical and genetic evidence indicates that ubiquitin-protein conjugates are essential intermediates for this proteolysis pathway [16]. Degradation by the ubiquitin system serves essential functions of the cellular stress response. Moreover, this pathway is involved in the control of the half-lives of important cellular regulators. The ubiquitin system mediates the selective degradation of the far-red light absorbing form of phytochrome (Pfr), a regulatory photoreceptor of higher plants [30]. Recent in vitro studies indicate that the short half-life of p53, a negative regulator of cell proliferation, can be controlled by the ubiquitin system [26]. In addition, a key step governing the exit from mitosis, degradation of the cell cycle regulator cyclin, is mediated by the ubiquitin pathway [11].

In this review we will focus on structural determinants for proteolysis and on a description of the enzymatic components of the ubiquitin system which perform a key function in selective protein degradation.

The ubiquitin system

Ubiquitin is a small, abundant and highly conserved intracellular protein. It has a tightly packed globular structure with three faces which might interact with other proteins: one acidic, one basic and one hydrophobic. The carboxyl terminus of ubiquitin, which protrudes from the globular core, becomes conjugated to substrate proteins. Ubiquitin conjugation is a multistep process (fig. 1). In an initial ATP-dependent step, which requires the activity of a *ubiquitin-activating enzyme*, E1, a thiolester is formed between the carboxyl-terminus of ubiquitin and a cysteine of the E1 enzyme. Subsequently, ubiquitin is transferred to a specific cysteine residue of a *ubiquitin-conjugating enzyme*, E2. This type of enzyme catalyzes the covalent attachment of ubiquitin to ε-amino groups of lysine residues of target proteins. All eukaryotes appear to have a large variety of these enzymes, which possess different substrate specificities and are involved in strikingly different cellular functions[19]. Some conjugation reactions require additional factors, known as 'ubiquitin-protein ligases' or E3s, to mediate substrate recognition and subsequent ubiquitination[6].

Degradation of ubiquitin-protein conjugates seems to be mediated by a multicatalytic protease complex (proteasome)[23]. This cylindrical particle appears to consist of several related but functionally distinct proteases and occurs both in the cytoplasm and the nucleus. Genes encoding proteasome subunits are essential for cell viability in yeast[10, 15].

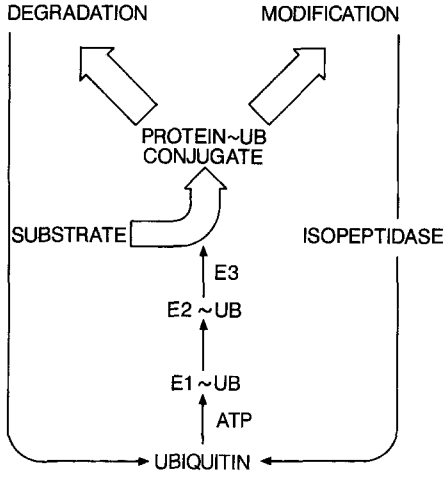

Figure 1. Ubiquitin conjugation pathway. Ubiquitin (UB) protein conjugation is catalyzed by ubiquitin-activating enzyme (E1) and a family of ubiquitin-conjugating enzymes (E2). The covalent attachment of ubiquitin to substrate proteins may lead to selective degration of the protein or may cause a modification of protein structure and function. Ubiquitin conjugation may be reversed by the action of isopeptidases which cleave ubiquitin from conjugates.

Function of ubiquitin-protein conjugation

According to an early hypothesis, ubiquitin in a protein conjugate serves as a tag marking proteins for degradation. However, the existence of metabolically stable ubiquitin conjugates indicates that ubiquitin may also have a function in the direct modification of protein structure and function. Such stable conjugates have been identified with proteins of the nucleus, the cytosol and the cell surface: chromosomal histones H2A and H2B[33], *Drosophila* actin[3], hormone receptors[22, 34], cell adhesion proteins[31] and viral coat proteins[8]. For some of these proteins ubiquitin conjugation may be reversible, since enzymes have been described which precisely cleave ubiquitin from conjugates[32].

In vivo, two types of ubiquitin conjugate structures have been observed. *Monoubiquitination* occurs at one or more lysine residues in target proteins. This type is typical for ubiquitin-histone conjugates which are metabolically stable. *Multiubiquitination* occurs when ubiquitin itself gets ubiquitinated at a specific lysine residue (lysine-48 of ubiquitin). Since such a branched multiubiquitin chain was discovered on a short-lived engineered protein, this structure was suggested to be a specific signal for protein degradation by the ubiquitin system[5]. Consistent with this hypothesis are experiments which showed that a ubiquitin variant, in which lysine-48 of ubiquitin was replaced by an arginine residue, failed to support both multiubiquitination and degradation of a test substrate. However, it remains to be shown whether in general the type of ubiquitin-protein structure determines whether a given protein will be degraded or not. Alternatively, the nature of the substrate itself might determine the structure and fate of the conjugate.

Signals for proteolysis

Selective protein degradation requires structural determinants on proteolytic substrates which target these proteins for destruction. These determinants can be recognized either directly by specific proteases or, alternatively, by specific recognition components of more complex proteolytic systems. Thus, the half-life of a protein in a given cellular environment is probably a function of the cellular concentration of these recognition components and their affinity to available target sites on proteins. Experimental evidence suggests that many proteins bear several distinct proteolytic determinants for different recognition components of proteolytic pathways[17]. Furthermore, long-lived proteins may be turned into proteins with short half-lives by structural alterations which expose cryptic signals. This might apply to the degradation of abnormal proteins and to the regulated degradation of proteins triggered by events which

influence protein structure such as light in the case of phytochrome [30].

Several approaches were taken to define amino acid determinants which affect protein stability. Based on a computer comparison of short-lived proteins a determinant that targets proteins for proteolysis has been suggested [25]. According to the so-called *PEST-hypothesis* proteins containing stretches of amino acids rich in proline, glutamic acid, serine, and threonine (P, E, S, T) are short-lived in vivo. However, a general function of PEST-sequences as proteolytic determinants is controversial and experiments addressing the validity of this hypothesis remain to be done.

One determinant for protein degradation by the ubiquitin system has been analyzed in detail in yeast: the *N-end rule*. In the case of a test protein, β-galactosidase, the identity of the amino-terminal amino acid determines the half-life of the protein [1]. Constructs bearing amino-terminal residues with bulky side chains (Met excluded; Arg, Lys, His, Phe, Trp, Tyr, Leu) are short-lived proteins in vivo. These amino acids are called *primary* destabilizing residues. Proteins with certain other amino-terminal residues can be turned into short-lived proteins by amino-terminal modifications. Amino-terminal Asn and Gln residues (*tertiary* destabilizing) are deamidated into Asp and Glu residues (*secondary* destabilizing) by specific enzymes. To these acidic amino-terminal residues Arg – a primary destabilizing residue – is added by a specific aminoacyl-tRNA protein transferase. Versions of the N-end rule seem to operate in various organisms [14].

Substrates of the N-end rule require a single lysine residue in close proximity to the amino-terminus for ubiquitination and subsequent degradation [2]. A recognition component, UBR1, recognizes destabilizing amino-terminal residues on a substrate. This protein is thought to interact with a ubiquitin-conjugating enzyme which adds ubiquitin to the acceptor lysine. In the case of β-galactosidase, a multiubiquitin chain is confined to this lysine residue [5]. The gene encoding the UBR1 protein has been cloned [4]. Overexpression of UBR1 protein results in an increased turnover of N-end rule constructs suggesting that UBR1 is a rate-limiting factor of this pathway. Although *ubr1* deletion mutants completely stabilize short-lived N-end rule substrates, the mutant has no obvious phenotype under various physiological conditions. This unexpected finding indicates that the N-end rule constitutes only one pathway for the degradation of proteins by the ubiquitin system.

Studies with certain N-end rule substrates led to implications for the proteolysis of oligomeric proteins. In a multisubunit protein complex the proteolytic determinant (e.g. a destabilizing residue of the N-end rule) and the actual acceptor lysine for ubiquitination may be located on different subunits [20]. In this case only the subunit which bears the lysine gets degraded. This mechanism of *cis-trans* recognition may apply to the regulated degradation of the tumor suppressor protein p53. Binding of the E6 protein of papilloma virus promotes the ubiquitination of p53. In such a complex only the p53 protein gets degraded [26].

Genetics of ubiquitin-dependent proteolysis

Genetic analysis of the ubiquitin system greatly improved our understanding of the in vivo function of the system. Analysis of the mutant mouse cell line ts85 with a thermolabile ubiquitin-activating enzyme demonstrated the essential character of ubiquitin-protein conjugation for cell viability. Moreover, these studies suggested that the majority of short-lived and abnormal proteins are degraded by the ubiquitin-dependent pathway [9].

A detailed molecular genetic analysis of ubiquitin-conjugating enzymes (UBC) has been performed in the yeast *Saccharomyces cerevisiae* [19]. This organism is uniquely suitable for investigations of the in vivo functions of gene products. These studies led to the discovery that the DNA repair gene *RAD6 (UBC2)* [18] and the cell cycle gene *CDC34 (UBC3)* [12] both encode ubiquitin-conjugating enzymes. Recently, the key components of ubiquitin-dependent proteolysis have been identified [27, 29]. Below, we will discuss the experiments which led to the functional characterization of these ubiquitin-conjugating enzymes.

Cloning and structure of UBC genes

Several enzymes of the yeast ubiquitin-protein ligase system have been purified by ubiquitin-Sepharose affinity chromatography. This purification scheme is based on ATP-dependent thiolester formation of these enzymes with immobilized ubiquitin, thereby exploiting their enzymatic activities. Using this method ubiquitin-activating enzyme, E1, and at least five distinct ubiquitin-conjugating enzymes, E2s, have been isolated (fig. 2). To clone the genes encoding these enzymes, two different strategies were followed. One approach involved the determination of partial amino acid sequences of the purified enzymes and synthesis of the corresponding oligonucleotides for use in library screening. This technique was applied to isolate *UBC4* and *UBC5* genes encoding $E2_{16K}$ enzymes (fig. 2). The alternative cloning strategy consisted of raising antibodies against the mixture of purified E1 and E2 enzymes and using this antiserum to screen a yeast expression library. Immunoreactive phage clones were identified which carried the gene *UBC1* encoding the $E2_{30K}$ ubiquitin-conjugating enzyme (fig. 2). In addition to structural criteria the identity of the isolated genes was verified by assaying enzymatic activity of the cloned gene products.

UBC4 and *UBC5* genes encode $E2_{16K}$ enzymes of almost identical amino acid sequence (137 identical residues out of a total of 148). Both genes contain a single intron located at identical positions in the amino-terminal part

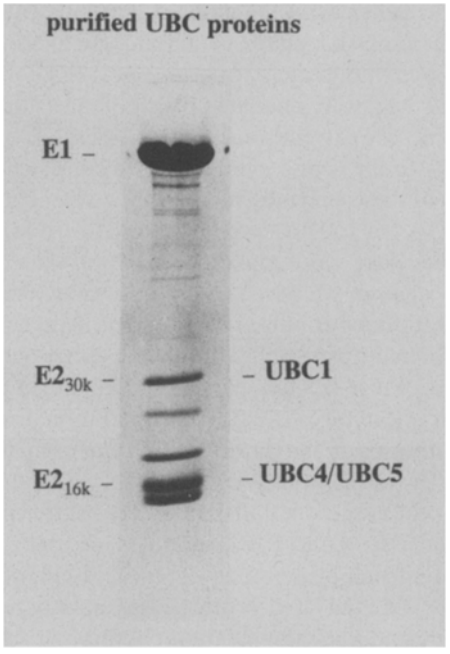

Figure 2. Purified components of the ubiquitin-protein ligase sytem. Ubiquitin-activating enzyme (E1) and a family of ubiquitin-conjugating enzymes (E2s) were affinity purified by covalent binding to immobilized ubiquitin (Coomassie stained polyacrylamide-SDS-gel). Yeast *UBC1* gene encodes $E2_{30k}$ enzyme, *UBC4* and *UBC5* genes encode $E2_{16k}$ enzymes.

of the coding region (fig. 3). Moreover, both genes are flanked by identical tRNA genes pointing to a gene duplication event which gave rise to *UBC4* and *UBC5* genes [28].

The predicted amino acid sequence of UBC1 shows that this enzyme is similar in sequence to all other characterized members of the ubiquitin-conjugating enzyme family. In particular, sequences surrounding the single active site cysteine residue required for ubiquitin-enzyme thiolester formation are highly conserved.Within a conserved domain common to all ubiquitin-conjugating enzymes about 45% of the amino acids are identical in UBC1 and UBC4/UBC5 proteins (fig. 3). UBC1 protein differs in its structure from UBC4/UBC5 in possessing a carboxyl-terminal extension. UBC4 and UBC5 have been defined as *class I* ubiquitin-conjugating enzymes which consist almost exclusively of the conserved "E2 domain". In comparison, UBC1 belongs to the *class II* E2 enzymes which bear carboxyl-terminal extensions. Both previously characterized class II enzymes, UBC2/RAD6 and UBC3/CDC34, have extensions which are known to be necessary for substrate interaction. Following this line, the carboxyl-terminal domain of the UBC1 protein, which is highly charged, may also be involved in substrate interaction. The class I enzymes UBC4 and UBC5 most likely require supplementing E3 factors for conjugation activity.

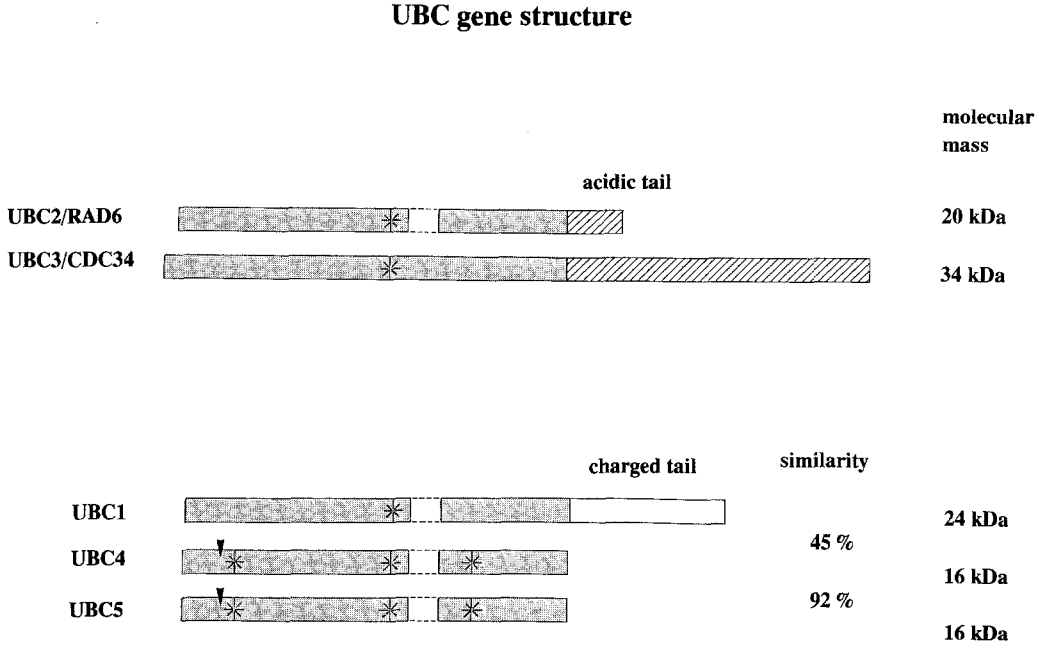

Figure 3. Schematic representation of the structure of yeast UBC gene products. Some ubiquitin-conjugating enzymes carry carboxyl-terminal extensions in addition to a conserved E2-domain (shaded region). The cysteine residues are indicated by asterisks. Intron positions in UBC4 and UBC5 genes are marked by arrows.

UBC1 functions during growth after a resting state

To assess the in vivo function of UBC1, UBC4 and UBC5 enzymes the corresponding genes were inactivated in yeast cells and the phenotypes of the resulting mutants were analyzed. *ubc1* disruption mutants displayed only a moderate slow growth phenotype. However, *ubc1* mutants were markedly impaired in growth after germination of spores. Tetrad analysis following sporulation of *ubc1/UBC1* heterozygotes showed that unlike wild-type spores, *ubc1* mutant spores gave rise to tiny, amorphously shaped colonies indicative of slow growth and poor cell viability at this stage of the yeast life cycle. This was in striking contrast to colonies formed by exponentially-growing haploid *ubc1* mutant cells, which had a normal appearance and were only slightly smaller than wild-type colonies. Interestingly, mating, sporulation, and germination of *ubc1* mutants appeared to be essentially unaffected. The specific requirement for UBC1 protein function upon resumption of growth following a resting state also manifested itself after prolonged nutrient deprivation or extended storage on plates. These data suggest that in addition to a role for exponentially growing cells, UBC1-mediated functions are critical for growth and viability at a specific point in the yeast life cycle: during the transition period after a state of quiescence.

UBC4 and UBC5 mediate the degradation of short-lived and abnormal proteins

Disruption of either one of the genes, *UBC4* or *UBC5*, caused only moderate defects. Disruption of both genes, however, resulted in severe phenotypes. This indicates that *UBC4* and *UBC5* genes have complementing cellular functions, as already suggested by the closely related amino acid sequences of the proteins. Growth of *ubc4ubc5* mutants was drastically impaired, double mutants formed cell aggregates, and individual cells were significantly larger than wild-type cells. This suggests that UBC4 and UBC5 enzymes mediate important cellular functions during vegetative growth.

By Western analysis using anti-ubiquitin antibodies it has been shown that UBC4 and UBC5 enzymes generate high molecular weight ubiquitin-protein conjugates in vivo. Moreover, the experimental data indicate that UBC4 and UBC5 enzymes comprise a major ubiquitin-conjugation activity in yeast cells.

Previous studies suggested that for certain proteins the attachment of a multiubiquitin chain to the proteolytic substrate might be required for protein degradation[5]. Synthesis of high molecular weight ubiquitin-protein conjugates by UBC4 and UBC5 enzymes indicated a function of these enzymes in selective protein degradation. To evaluate this possibility the sensitivity of mutants to canavanine was analyzed. Cells incubated with canavanine, an amino acid analog, generate abnormal proteins in vivo which are usually degraded rapidly. Wild-type cells, as well as *ubc5* mutants, efficiently formed colonies on plates containing canavanine. *ubc4* mutants were moderately sensitive. *ubc4ubc5* double mutants, however, were unable to form colonies under these conditions. The extreme sensitivity of UBC4/UBC5 deficient cells to an amino acid analog is consistent with a function of these enzymes in the elimination of abnormal proteins.

The turnover of naturally short-lived proteins or canavanyl-proteins in wild-type and mutant cells was directly analyzed. Following pulse-labeling of proteins the release of radioactivity from cells during the chase period was measured. Pulse-labeled proteins were degraded efficiently in wild-type cells. Protein degradation in *ubc4* and *ubc5* mutants was barely affected. In *ubc4ubc5* double mutants, however, turnover of short-lived proteins was significantly reduced to about half the wild-type value. Moreover, UBC4 and UBC5 enzymes mediated proteolysis of abnormal proteins: in *ubc4ubc5* double mutants, proteolysis of canavanyl-proteins was severely restricted. Even *ubc4* and *ubc5* single mutants exhibited significant defects. The function of UBC4 and UBC5 enzymes in protein degradation was specific for short-lived and abnormal proteins: neither the turnover of long-lived proteins, nor the rate of labelled amino acid uptake or protein synthesis, was affected.

UBC4 and UBC5 are involved in the cellular stress response

The expression of genes encoding components of the ubiquitin system appears to be highly regulated. Therefore the expression of *UBC* genes was examined under different growth conditions and in response to environmental stress. On Northern blots with total yeast RNA, *UBC1* transcripts were detected in exponentially growing cells. *UBC1* transcription increased substantially when the cells entered the stationary phase. *UBC1* expression was unaffected by a heat-shock treatment. *UBC4* expression was high in growing cells but hardly detectable during transit to the stationary phase. In contrast, *UBC5* was only weakly expressed during exponential growth but was highly induced upon entry into the stationary phase. Interestingly, expression of *UBC4* and *UBC5* increased substantially in response to heat shock.

Given the heat-inducible expression of *UBC4* and *UBC5* genes, the resistance of mutants to chronic heat stress was investigated. *ubc4ubc5* mutants were non-viable at elevated temperature. The thermosensitivity of the growth of *ubc4ubc5* mutants was not merely due to growth arrest but was caused by cell death. UBC4 and UBC5 proteins apparently fulfil essential functions under stress conditions which are thought to generate abnormal proteins, such as elevated temperatures or the presence of an amino acid analog.

Wild-type yeast cells are sensitive to an acute heat shock, and several stress conditions are known to induce re-

sistance to an acute heat shock. In contrast to single mutants and wild-type cells, *ubc4ubc5* double mutants exhibited a striking level of constitutive thermotolerance which suggest that the stress response is turned on in *ubc4ubc5* mutants at normal temperatures. Further support for this suggestion came from the analysis of heat-shock protein expression in *ubc4ubc5* mutant cells. In these mutants cells, hsp70 stress proteins, which appeared in wild-type cells only upon heat shock, were synthesized already at normal growth temperatures. This indicates that the loss of UBC4/UBC5 function which causes pronounced deficiencies in selective protein degradation induces constitutive expression of heat-shock proteins. These results are consistent with previous observations in other systems that abnormal proteins act as inducers of a heat shock-like response. Since abnormal proteins are eliminated by a stress-inducible proteolytic pathway, a feed-back control of the stress response has been suggested.

In *E. coli* the activity of a single enzyme, the *lon* gene product, functionally resembles the much more complex ubiquitin-dependent proteolytic system of eukaryotes. The *lon* gene encodes an ATP-dependent protease specific for abnormal and short-lived proteins, and its expression is induced by heat shock and other stresses [13].

UBC1, UBC4 and UBC5 constitute a UBC-subfamily essential for cell viability

To evaluate a possible involvement of UBC1 in ubiquitin-mediated protein turnover, the sensitivity of *ubc1* mutants to the amino acid analog canavanine was examined. When compared to congenic wild-type cells, *ubc1* mutants were more sensitive to canavanine, which suggests that UBC1 might function as an enzymatic component of the ubiquitin-mediated pathway for selective protein degradation. This conclusion was further supported by direct measurements of the turnover of canavanyl-proteins, which showed a slight but significant reduction of selective protein degradation in *ubc1* mutants.

The moderate defects in protein degradation observed in *ubc1* mutants might indicate a functional overlap of UBC1 with the UBC4 and UBC5 enzymes which mediate most of the ubiquitin-dependent protein degradation in yeast. To address this possibility, double and triple mutants in these *UBC* genes were constructed. Disruption of *UBC1* in a *ubc4* mutant background resulted in double mutants exhibiting more severe phenotypes than that of the single mutants combined. In particular, *ubc1ubc4* double mutants showed significantly prolonged doubling times, suggesting important overlapping functions for these two genes during exponential growth. Moreover, these studies confirmed the critical role of UBC1 after sporulation and germination: *ubc1ubc4* mutants spores failed to resume growth after germination. One explanation for this phenotype is that certain proteins have to be degraded by a UBC1-mediated pathway before cells resume growth. *ubc1ubc5* double mutants showed no apparent phenotypic difference from *ubc1* single mutants. This is consistent with the previous observation that *UBC5* is dispensable as long as *UBC4* is present. By several genetic criteria it was demonstrated that the combined loss-of-function of these three *UBC* genes resulted in inviable cells, indicating that *UBC1*, *UBC4* and *UBC5* genes constitute a subfamily of *UBC* genes essential for cell viability.

In *ubc4ubc5* mutants, the overexpression of *UBC1* improved growth. In particular, high level expression of *UBC1* restored growth of *ubc4ubc5* mutants at elevated temperatures and increased resistance of these mutants to canavanine. Apparently, the defects of *ubc4ubc5* mutants in the ubiquitin-mediated proteolysis pathway can be complemented by overexpression of UBC1.

In conclusion, ubiquitin-conjugating enzymes UBC1, UBC4 and UBC5 from the yeast *Saccharomyces cerevisiae* have been characterized. These proteins were found to be key enzymes of the ubiquitin-mediated proteolytic system, which apparently catalyze the attachment of ubiquitin to proteolytic substrates. Genetic analysis has shown that UBC1, UBC4, and UBC5 enzymes have overlapping functions and constitute a UBC subfamily essential for cell viability: single genes are dispensable but deletion of all three genes renders cells inviable. These data suggest that ubiquitin-mediated protein degradation is a vital function of eukaryotic cells. Despite structural and functional similarities, these enzymes are involved in distinct cellular processes. UBC1 is specifically required for resuming growth after a resting state. The closely related enzymes UBC4 and UBC5 perform important functions during mitotic growth and become essential for cell viability under stress conditions.

One striking feature of intracellular protein degradation is its exceptional selectivity. The observed enzymatic diversity at the level of ubiquitin-conjugation may contribute to the precise regulation and specificity of this process.

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1 Bachmair, A., Finley, D., and Varshavsky, A., *In vivo* half-life of a protein is a function of its amino-terminal residue. Science *234* (1986) 179–186.
2 Bachmair, A., and Varshavsky, A., The degradation signal in a short-lived protein. Cell *56* (1989) 1019–1032.
3 Ball, E., Karlik, C. C., Beall, C. J., Saville, D. L., Sparrow, J. C., Bullard, B., and Fyrberg, E. A., Arthrin, a myofibrillar protein of insect flight muscle, is an actin-ubiquitin conjugate. Cell *51* (1987) 221–228.
4 Bartel, B., Wünning, I., and Varshavsky, A., The recognition component of the N-end rule pathway. EMBO J. *9* (1990) 3197–3189.
5 Chau, V., Tobias, J. W., Bachmair, A., Marriott, D., Ecker, D. J., Gonda, D. K., and Vashavsky, A., A multiubiquitin chain is confined to specific lysine in a targeted short-lived protein. Science *234* (1989) 1576–1583.
6 Ciechanover, A., and Schwartz, A. L., How are substrates recognized by the ubiquitin-mediated proteolytic system? Trends biochem. Sci. *14* (1989) 483–488.
7 Dice, J. F., Peptide sequences that target cytosolic proteins for lysosomal proteolysis. Trends biochem. Sci. *15* (1990) 3505–309.

8 Dunigan, D. D., Dietzgen, R. G., Schoelz, J. E., and Zaitlin, M., Tobacco mosaic virus particles contain ubiquitinated coat protein subunits. Virology *165* (1988) 310–312.
9 Finley, D., and Varshavsky, A., The ubiquitin system: functions and mechanisms. Trends biochem. Sci. *10* (1985) 343–346.
10 Fujiwara, T., Tanaka, K., Orino, E., Yoshimura, T., Kumatori, A., Tamura, T., Chung, C. H., Nakai, T., Yamaguchi, K., Shin, S., Kakizuka, A., Naknishi, S., and Ichihara, A., Proteasomes are essential for yeast proliferation. J. biol. Chem. *265* (1990) 16604–16613.
11 Glotzer, M., Murray, A. W., and Kirschner, M. W., Cyclin is degraded by the ubiquitin pathway. Nature *349* (1991) 132–138.
12 Goebl, M. G., Yochem, J., Jentsch, S., McGrath, J. P., Varshavsky, A., and Byers, B., The yeast cell cycle gene *CDC34* encodes a ubiquitin-conjugating enzyme. Science *241* (1988) 1331–1335.
13 Goff, S. A., and Goldberg, A. L., Production of abnormal proteins in *E. coli* stimulates transcription of *lon* and other heat shocks genes. Cell *41* (1985) 587–595.
14 Gonda, D. K., Bachmair, A., Wünning, I., Tobias, J. W., Lane, W. S., and Varshavsky, A., Universality and structure of the N-end rule. J. biol. Chem. *264* (1989) 16700–16712.
15 Heinemeyer, W., Kleinschmidt, J. A., Saidowsky, J., Escher, C., and Wolf, D. H., Proteinase yscE, the yeast proteasome/multicatalytic-multifunctional proteinase: mutants unravel its function in stress induced proteolysis and uncover its necessity for cell survival. EMBO J. *10* (1991) 555–562.
16 Hershko, A., Ubiquitin-mediated protein degradation. J. biol. Chem. *263* (1988) 15237–15240.
17 Hochstrasser, M., and Varshavsky, A., In vivo degradation of a transcriptional regulator: the yeast α2-repressor. Cell *61* (1990) 697–708.
18 Jentsch, S., McGrath. J. P., and Varshavsky, A., The yeast DNA repair gene *RAD6* encodes a ubiquitin-conjugating enzyme. Nature *329* (1987) 131–134
19 Jentsch, S., Seufert, W., Sommer, T., and Reins, H.-A., Ubiquitin-conjugating enzymes: novel regulators of eukaryotic cells. Trends biochem. Sci. *15* (1990) 195–198.
20 Johnson, E. S., Gonda, D. K., and Varshavsky, A., Cis-trans recognition and subunit-specific degradation of short-lived proteins. Nature *346* (1990) 287–291.
21 Klausner, R. D., and Sitia, R., Protein degradation in the endoplasmic reticulum. Cell *62* (1990) 611–614.
22 Leung, D. W., Spencer, S. A., Cachianes, G., Hammonds, R. G., Collins, C., Menzel, W. J., Barnard, R., Waters, W. J., and Wood, W. I., Growth hormone receptor and serum binding protein: purification, cloning and expression. Nature *330* (1987) 537–543.
23 Matthews, W., Tanaka, K., Driscoll, J., Ichihara, A., and Goldberg, A. L., Involvement of the proteasome in various degradative processes in mammalian cells. Proc. natl Acad. Sci. USA *86* (1989) 2597–2601.
24 Rechsteiner, M., Ubiquitin-mediated pathways for intracellular proteolysis. Ann. Rev. cell. Biol. *3* (1987) 1–30.
25 Rogers, S., Wells, R., and Rechsteiner, M., Amino acid sequences common to rapidly degraded proteins: the PEST hypothesis. Science *234* (1986) 364–368.
26 Scheffner, M., Werness, B. A., Huibregtse, J. M., Levine, A. J., and Howley, P. M., The E6 oncoprotein encoded by human papillomavirus types 16 and 18 promotes the degradation of p53. Cell *63* (1990) 1129–1136.
27 Seufert, W., and Jentsch, S., Ubiquitin-conjugating enzymes UBC4 and UBC5 mediate selective degradation of short-lived and abnormal proteins. EMBO J. *9* (1990) 543–550.
28 Seufert, W., and Jentsch, S., Nucleotide sequence of two $tRNA^{Arg}$-$tRNA^{ASP}$ tandem genes linked to duplicated *UBC* genes in *Saccharomyces cerevisiae*. Nucl. Acids Res. *18* (1990) 1638
29 Seufert, W., McGrath, J. P., and Jentsch, S., *UBC1* encodes a novel member of an essential subfamily of yeast ubiquitin-conjugating enzymes involved in protein degradation. EMBO J. *9* (1990) 4535–4541.
30 Shanklin, J., Jabben, M., and Vierstra, R. D., Red light-induced formation of ubiquitin-phytochrome conjugates: identification of possible intermediates of phytochrome degradation. Proc. natl Acad. Sci. USA *84* (1987) 359–363.
31 Siegelman, M., Bond, M. W., Gallatin, W. M., St. John, T., Smith, H. T., Fried, V. A., and Weissman, I. L. Cell surface molecule associated with lymphocyte homing is a ubiquitinated branched-chain glycoprotein. Science *231* (19876) 823–829.
32 Wilkinson, K. D., Lee, K., Deshpande, S., Duerksen-Hughes, P., Boss, J. M., and Pohl, J., The neuron-specific PGP 9.5 is a ubiquitin carboxyl-terminal hydrolase. Science *246* (1989) 670–673.
33 Wu, R. S., Kohn, K. W., and Bonner, W. M., Metabolism of ubiquitinated histones. J. biol. Chem. *256* (1981) 5916–5920.
34 Yarden, Y., Escobedo, J. A., Kuang, W. J., Yang-Feng, T. L., Daniel, T. O., Tremble, P. M., Chen, E. Y., Ando, M. E., Harkins, R. N., Francke, U., Fried, V. A., Ullrich, A., and Williams, L. T., Structure of the receptor for platelet-derived growth factor helps define a family of closely related growth factor receptors. Nature *323* (1986) 226–232.

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Proteases and protein degradation in *Escherichia coli*

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Abstract. In *E. coli*, protein degradation plays important roles in regulating the levels of specific proteins and in eliminating damaged or abnormal proteins. *E. coli* possess a very large number of proteolytic enzymes distributed in the cytoplasm, the inner membrane, and the periplasm, but, with few exceptions, the physiological functions of these proteases are not known. More than 90% of the protein degradation occurring in the cytoplasm is energy-dependent, but the activities of most *E. coli* proteases in vitro are not energy-dependent. Two ATP-dependent proteases, Lon and Clp, are responsible for 70–80% of the energy-dependent degradation of proteins in vivo. In vitro studies with Lon and Clp indicate that both proteases directly interact with substrates for degradation. ATP functions as an allosteric effector promoting an active conformation of the proteases, and ATP hydrolysis is required for rapid catalytic turnover of peptide bond cleavage in proteins. Lon and Clp show virtually no homology at the amino acid level, and thus it appears that at least two families of ATP-dependent proteases have evolved independently.
Key words. ATP-dependent; degradation; protease; Lon; Clp.