Lysosomal and non-lysosomal proteolysis in the eukaryotic cell: studies on yeast

Wolfgang Heinemeyer, Angela Simeon, Hans H. Hirsch, Hans H. Schiffer, Ulrich Teichert and Dieter H. Wolf
Institut für Biochemie der Universität Stuttgart, Pfaffenwaldring 55, W-7000 Stuttgart 80, Germany

Introduction

Proteolysis is an essential tool in regulating cellular functions at the post-translational level. The yeast *Saccharomyces cerevisiae* serves as an excellent model organism for studying the biological role of proteolysis in the eukaryotic cell as it is accessible to the combination of biochemical, genetic and molecular biological techniques [1–3]. As found for mammalian cells, two major proteolytic systems involved in the degradation of proteins have been found in yeast up to now: a lysosomal (vacuolar) system and a cytoplasmic system [4, 5]. The lysosomal system is composed of two endopeptidases and five exopeptidases [3–5]. The cytoplasmic system involved in protein degradation, elucidated so far, consists of proteinase yscE, the equivalent of the mammalian proteasome/multicatalytic-multifunctional proteinase [6, 7]. Here we report on the function of both the lysosomal and the cytoplasmic proteolytic system in cellular life.

Results

The lysosomal proteolytic system of the yeast *S. cerevisiae* consists of two endopeptidases; proteinase yscA, an acid proteinase equivalent to cathepsin D of mammalian cells, and proteinase yscB, a serine proteinase; two carboxypeptidases, carboxypeptidase yscY and yscS; two aminopeptidases, aminopeptidase ysc1 and yscCo, as well as dipeptidylaminopeptidase yscV [4, 5]. The isolation of mutants deficient in the activities of several of the lysosomal peptidases uncovered their cellular function. The two endopeptidases, proteinase yscA and yscB, are responsible for about 85% of protein degradation under nutritional stress conditions. Under these conditions proteinase yscA – not proteinase yscB – proved to be vital for cellular life: Shift from rich to poor medium lacking any one of the essential nutrients carbon, nitrogen, phosphate etc. leads to cell death [8]. Proteinase yscA-deficient cells are also sensitive to the application of canavanine, an inducer of synthesis of false proteins (V. Möhrle, unpublished work). Absence of either of the two lysosomal endopeptidases or a combination of endopeptidase and the two lysosomal carboxypeptidases results in a disturbed differentiation process of sporulation in diploid cells.

Abbreviation used: ER, endoplasmic reticulum.

The highly unspecific character of the two lysosomal endopeptidases, proteinase yscA and proteinase yscB, *in vivo* is also reflected by their action *in vitro*: Both enzymes together degrade more than 85% of [^{3}H]methylated yeast proteins or more than 97% of [^{3}H]methylated casein in the test tube [8]. This highly unspecific character of both endopeptidases is opposed by their rather specific action in the maturation events of lysosomal proteinases: Proteinase yscA, proteinase yscB and carboxypeptidase yscY are synthesized as higher molecular mass precursors, which travel through the secretory pathway [endoplasmic reticulum (ER), Golgi] to the lysosome (vacuole) [5, 9]. Proteinase yscA is synthesized as an approximately 52 kDa precursor which undergoes maturation to the 42 kDa mature product [5, 9–12]. This maturation step is thought to be autocatalytic when the protein enters the lysosome (vacuole) [11, 12]. Proteinase yscB is synthesized as a high molecular mass precursor of 73 kDa [13, 14] which undergoes two proteolytic maturation steps: The first step involves cleavage of the highly charged *N*-terminal 'super'-peptide upon entry of the enzyme into the ER [14, 15]. Active site mutagenesis of proteinase yscB indicates this step to be of autocatalytic nature (H. H. Hirsch, H. H. Schiffer & D. H. Wolf, unpublished work). The second maturation step of the resulting pro-proteinase yscB of approximately 42 kDa occurs upon entry into the lysosome. It involves an initiating cleavage step by proteinase yscA followed by further self-maturation of proteinase yscB to the 33 kDa enzyme [14]. Carboxypeptidase yscY maturation is also proteinase yscA-dependent [11, 12, 16], but needs proteinase yscB cleavage for appearance of its authentic mature form [16]. There is indication that proteinase yscA is only the trigger in the maturation events occurring upon entry of the enzymes into the lysosome (vacuole), initiating maturation of proteinase yscB, and that proteinase yscB is the actual maturating enzyme (H. H. Hirsch, H. H. Schiffer & D. H. Wolf, unpublished work).

While knocking out the lysosomal endopeptidases leads to a dramatic loss of protein degradation capacity in nutritionally stressed cells, protein degradation in actively growing cells remains intact to a major extent [8]. We had found and purified a prominent non-lysosomal proteinase in lysosomal proteinase-deficient cells, which we had called pro-

teinase yscE [6]. The enzyme turned out to be the equivalent of the mammalian proteasome/multicatalytic-multifunctional proteinase [7]. The enzyme carries three distinct proteolytic activities: a chymotrypsin-like, a trypsin-like and a peptidyl glutamyl-peptide-splitting activity [17, 18]. Mutation of the chymotryptic activity of the high molecular mass, multisubunit enzyme uncovered its function in the ubiquitin-mediated proteolytic pathway [18] which is thought to be of cytoplasmic nature: Protein degradation is considerably disturbed in temperature and canavanine-stressed mutant cells, canavanine sensitivity is enhanced and mutant cells accumulate ubiquitinated proteins [18]. Lack of a subunit of the multisubunit enzyme is not tolerated by the cell: Null mutant cells of different subunits of proteinase yscE are unable to survive [18–20]. This behaviour might be due to the complete shut off of all proteasomal functions. One such function might be the destruction of rapidly turned over, short-lived signalling proteins such as cyclin, a component of cell cycle regulation [21]. This protein has been shown to be degraded via the ubiquitin pathway [21]. Nuclear targeting signals have been shown to be located on subunits of the proteasome [18–20].

Ubiquitin-mediated proteolysis has long been thought to be a non-lysosomal event [22]. Yeast cells deficient in the two lysosomal (vacuolar) endopeptidases, proteinase yscA and proteinase yscB, show a dramatically altered lysosome and accumulate proteins in this organelle. A considerable amount of the accumulated proteins is ubiquitinated (A. Simeon & D. H. Wolf, unpublished work). This observation links ubiquitin also to lysosomal protein degradation.

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