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Proteinases, Proteolysis and Biological Control in the Yeast *Saccharomyces cerevisiae*

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INTRODUCTION

The vital role proteolysis plays in the control of cellular events has become fully evident in recent years.[1–5] Understanding of cellular control through action of proteinases depends on our knowledge of the proteinases involved, the processes which are proteolytically catalysed and the signals which render a protein accessible to cleavage of one or more of its peptide bonds by a certain proteinase. The easy accessibility of the yeast *Saccharomyces cerevisiae* to a combined biochemical and genetic approach has made this organism a pacemaker in studying intracellular control through action of proteinases in the eukaryotic cell. A number of reviews on proteolysis in yeast have been published in the past five years[2,6–8] reflecting the speed with which this field has progressed. For additional information these articles should be consulted. The reader of the article presented here will soon recognize that the rapid progress in the field of proteinases, proteolysis and biological control in the yeast *Saccharomyces cerevisiae* continues.

All compartments of the cell (vacuoles, mitochondria, nucleus, endoplasmic reticulum. Golgi

apparatus, secretory vesicles, plasma membrane, cytoplasm, periplasm) are possible locations for proteinases and possible sites for proteolysis. In the text of this article the known proteinases of the yeast cell will be listed after their intracellular localization. In the Table they will be listed according to their endo- or exo-peptidolytic specificity. Known proteolytic processes will be described and, where possible their mechanism will be discussed. In several cases we already have a very precise idea about a proteolytic process and the catalyst involved as a result of genetic studies. Mutant strains that lack specifically one or more of the proteolytic enzymes provide the important link between the *in vitro* analysis of the proteolytic activity and the biological events catalysed by this activity. Such mutant strains also made possible the recent detection of a multiplicity of new proteinases in the yeast cell.

PROTEINASES

Nomenclature

Proteinases of *Saccharomyces cerevisiae* have been named with letters (endoproteinases, carboxypeptidases) and with roman numbers (aminopeptidases) (for proteinases A and B see references 9, 10; for carboxypeptidase Y see reference 11; for carboxypeptidase S see reference 12; for aminopeptidases I, II and III see reference 13).

However, this nomenclature has also been used for the increasing number of proteinases of other organisms without paying attention to the fact that enzymes with the same name mostly do not share common characteristics (for reviews and references see 2,3). To distinguish easily between the yeast proteinases and proteinases with identical names from other organisms we introduced the addition of the first letters of the organism (yeast), the genus (Saccharomyces) and the species (cerevisiae) to the name of the proteinase.[14]

Vacuolar proteinases

The vacuole has been designated as the digestive compartment of the yeast cell[15,16] and thus is, at least in part, functionally related to the lysosome of higher eukaryotic organisms. The vacuole contains the proteinases which were first detected and characterized in yeast[9,10,16-19] and which, for a long time, remained the only well known yeast proteinases (for reviews see references 2, 6). This was mainly due to the fact that these enzymes could readily be detected *in vitro* by using highly unspecific substrates like collagen derivatives, casein or haemoglobin.

Proteinase yscA. Proteinase yscA is a soluble, carboxylic endopeptidase;[9,10,20-22] (for classification of proteinases see reference 3). The enzyme consists of a single polypeptide chain of M_r of about 42 000 which carries about 8·5% carbohydrate.[21,23] Two carbohydrate chains seem to be present and they appear to be asparagine linked, since glycosylation is prevented when tunicamycin is added to cells.[23] The enzyme can be assayed using acid denatured haemoglobin[9,21] or casein[21] with pH optima of 3 and 6, respectively. The enzyme can also be measured using the fluorogenic peptide succinyl-Arg-Pro-Phe-His-Leu-Leu-Val-Tyr-7-amino-4-methyl-coumarin as substrate at about pH 5. Hydrolysis occurs at the Leu-Val bond.[24] The enzyme is inhibited by pepstatin, diazoacetyl-D,L-norleucine methylester and by 1,2-epoxy-3-(4-nitrophenoxy) propane.[21] Proteinase yscA activity is partly cryptic in whole-cell extracts.[25] This is due to a proteinase yscA-specific cytoplasmic inhibitor protein (I_3^A) of M_r of about 7700.[19,25-28] Incubation of cell extracts at low pH releases proteinase yscA activity from inhibition most likely due to digestion of I_3^A by proteinase yscB.[26,29] Activity levels of proteinase yscA increase several fold *in vivo* when cells enter diauxic growth and stationary phase, grow on acetate or are transferred onto nitrogen-free medium.[30-33]

Proteinase yscB. Proteinase yscB is a soluble serine-, sulphydryl endopeptidase exhibiting an activity optimum in the neutral pH range.[9,10,20,34-37] It consists of a single polypeptide chain of M_r of about 33 000.[23,36] The enzyme was reported to contain about 10% carbohydrate.[36] Binding of this carbohydrate is, however, not sensitive to endoglycosidase H treatment *in vitro* nor to tunicamycin treatment of cells *in vivo*[23] indicating some linkage other than to asparagine. The enzyme can be assayed with the chromophoric collagen derivatives Azocoll[34,38,39] and Hide Powder Azure.[34] In accordance with its low specificity the enzyme splits urea-denatured proteins, gelatin,[10] haemoglobin and casein.[9,10] Chromogenic peptides as are succinyl-Ala-Ala-Pro-Phe-4-nitroanilide,[40] benzoyl-Tyr-4-nitroanilide as well as ester substrates of trypsin and chymotrypsin[37] are also cleaved. The enzyme is strongly inhibited by the serine modifying agents diisopropylfluorophosphate[9,36] and phenylmethylsulphonyl fluoride.[36] Mercury com-

pounds are also strong inhibitors of proteinase yscB,[9,34,36] as is the actinomycete inhibitor chymostatin.[34] Proteinase yscB activity is cryptic in whole cell extracts. This phenomenon is brought about by a proteinase yscB-specific cytoplasmic inhibitor protein (I_2^B) of M_r of about 8500.[27,41-43] Incubation of cell extracts at low pH[25] or with 0·05 to 0·65% sodium dodecyl sulphate at neutral pH[44-46] or with pepsin[46] releases proteinase yscB activity from inhibition. Activity levels of proteinase yscB increase several fold *in vivo* when cells enter diauxic growth and stationary phase, grow on acetate or are transferred onto nitrogen-free medium.[31-33]

Carboxypeptidase yscY. Carboxypeptidase yscY is the most extensively studied peptidase in *Saccharomyces cerevisiae*.[9,11,20,47-58] It is a soluble serine exopeptidase active in the slightly acidic to neutral pH range [47,49,50,55] consisting of one polypeptide chain of M_r of about 61 000.[49,55] The enzyme has been sequenced.[57,58] The enzyme carries four asparagine-linked oligosaccharide chains, three of which have an average composition of $GlcNAc_2$ Man_{16} and carry phosphate groups.[59-64] The fourth chain is phosphate-free and has an average composition of $GlcNAc_2$ Man_{10}.[64] The enzyme exhibits a broad specificity against peptides, aminoacyl-esters and amides as well as against the chromogenic substrate benzoyl-Tyr-4-nitro-anilide (for review see reference 55). It also attacks proteins.[47,55] The enzyme is strongly inhibited by the serine residue modifying agents, diisopropylfluorophosphate and phenylmethylsulphonyl fluoride. Benzyloxycarbonyl-L-phenylalanine chloromethyl ketone inactivates the enzyme by alkylating a single histidine residue necessary for activity. Mercurials inhibit carboxy-peptidase yscY activity by binding to a single SH-group, which is, however, no part of the active centre (for review see reference 55). A specific polypeptide inhibitor (I^C) of carboxypeptidase yscY of M_r of about 25 000 is located in the cytoplasm of the yeast cell.[27,65] Activity levels of carboxypeptidase yscY increase several fold *in vivo,* when cells enter diauxic growth and stationary phase, grow on acetate, grow on a peptide, which is a substrate for the enzyme or are transferred onto nitrogen-free medium.[31,33,66]

Carboxypeptidase yscS. In a mutant lacking carboxypeptidase yscY a second vacuolar carboxypeptidase, called carboxypeptidase yscS, was detected.[12,67] The enzyme cleaves benzyloxycarbonyl-glycyl-leucine with high efficiency.[12] The metal ion chelating agent EDTA is a potent inhibitor or carboxypeptidase yscS, indicating that the enzyme is a metal ion-dependent peptidase. Zn^{2+} ions completely restore activity.[12] Activity levels of carboxypeptidase yscS are regulated by the nitrogen source. Highest activity can be achieved when cells are grown on benzyloxycarbonyl-glycyl-leucine as sole nitrogen source.[66]

Aminopeptidase yscI. The vacuolar aminopeptidase yscI[68] is a soluble metalloexopeptidase of 640 000 molecular weight.[13] Aminopeptidase yscI activity can be measured in the slightly basic pH range using leucine-4-nitroanilide or alanine-4-nitroanilide.[13] The enzyme is also active on a variety of peptides such as Leu-Gly, Leu-Leu, Leu-Val, Leu-Gly-Gly, Ala-Thr-Ala and leucineamide.[13] The enzyme is strongly inhibited by metal chelating agents.[13]

Aminopeptidase yscCo. Aminopeptidase yscCo is a soluble metallo-exopeptidase of M_r of about 100 000.[67,70] The enzyme is active only in the presence of small amounts of Co^{2+}-ions.[70] The enzyme cleaves arginine-4-nitroanilide and lysine-4-nitro-anilide with high efficiency. pH optimum is in the moderately alkaline range.[70] The enzyme is strongly inhibited by EDTA and Zn^{2+} ions. The actinomycete inhibitor bestatin which inhibits most yeast aminopeptidases tested so far[69] is not effective on aminopeptidase yscCo.[70] Aminopeptidase yscCo activity dramatically increases when cells reach stationary growth phase.[71]

Dipeptidyl aminopeptidase yscV. Dipeptidyl aminopeptidase yscV is an exopeptidase associated with the vacuolar membrane.[72] A molecular weight of 40 000 was found for the peptide chain under denaturing conditions.[73] The enzyme cleaves alanyl-prolyl-4-nitroanilide at the 4-nitroanilide bond in the neutral pH range. In general, the enzyme specifically hydrolyses peptide bonds involving the carboxyl group of prolyl residues penultimate to unprotected amino termini, unless arginine is the N-terminal amino acid. The enzyme is strongly inhibited by phenylmethylsulphonyl fluoride indicating a serine residue in the active centre. Heavy metal ions were also found to inhibit the enzyme.[73]

Other vacuolar proteinases. Cell fractionation studies using a mutant devoid of four vacuolar

enzymes, the two endoproteinases yscA and yscB and the two carboxypeptidases yscY and yscS, uncovered additional proteolytic activity in the vacuole. Most pronounced activity was found against the two chromogenic peptide substrates benzoyl-Ile-Glu-Gly-Arg-4-nitroanilide and tosyl-Gly-Pro-Arg-4-nitroanilide splitting at a site other than the 4-nitroanilide bond.[67] The number of proteinases represented by this activity is unknown.

Mitochondrial proteinases

Matrix located proteinase—proteinase yscMpI. The matrix located proteinase—for reasons of a unifying nomenclature here called proteinase yscMpI [Mp, mitochondria (precursor) processing]—is a soluble metalloendopeptidase of M_r of about 115 000.[74-75] The enzyme is only active against precursor forms of nuclear coded proteins which are imported into the mitochondrion. Precursors of proteins destined for the mitochondrial matrix, inner membrane and intermembrane space are cleaved.[74-76] The proteinase activity has a pH optimum in the neutral range and is inhibited by the metal chelators 1,10-phenanthroline and EDTA. Zn^{2+}, Co^{2+} and Mn^{2+}-ions restore the enzyme activity.[75,76]

Other mitochondrial proteinases. Three proteolytic activities of M_r of about 17 000, located in the inner mitochondrial membrane and active against cytochrome *c* as substrate have been described. Hydrolytic activity against cytochrome *c* is sensitive to inhibition by phenylmethylsulphonyl fluoride, *p*-chloromercuriphenyl sulphonate and leupeptin. In addition, the presence of three proteins active against benzoyl-DL-arginine-β-naphthylamide and benzoyl-DL-arginine-4-nitroanilide were reported.[77]

Periplasmic space proteinases

Aminopeptidase yscII. Aminopeptidase yscII is a soluble metallo-exopeptidase most active in the neutral pH range.[13,78] The molecular weight of the glycoprotein was reported to range from 85 000 to 140 000, the differences being attributed to varying degrees of glycosylation.[13,79,80] Activity of the enzyme can be assayed using a variety of different aminoacyl-4-nitroanilides, aminoacyl-β-naphthylamides, dipeptides and tripeptides.[13,69,79,80] The enzyme is strongly inhibited by EDTA, bestatin and Zn^{2+} and Hg^{2+}-ions at higher concentrations.[69,79,80]

Proteinases of unknown localization—soluble proteinases

Proteinase yscD. Proteinase yscD is a metallo sulphydryl endopeptidase. It consists of a single polypeptide chain of M_r of about 83 000.[81] The enzyme is not localized in the vacuole of the yeast cell.[67] Proteinase yscD cleaves the Pro-Phe bond of the synthetic peptide substrate benzoyl-Pro-Phe-Arg-4-nitroanilide and the Ala-Ala-bond of acetyl-Ala-Ala-Pro-Ala-4-nitroanilide, acetyl-Ala-Ala-Pro-Phe-4-nitroanilide and methoxysuccinyl-Ala-Ala-Pro-Met-4-nitroanilide with high efficiency. pH optima for cleavage range from slightly acidic to neutral. Mercurials and EDTA are potent inhibitors of proteinase yscD.[81]

Proteinase yscE. Proteinase yscE is a sulphydryl endopeptidase of M_r of about 600 000 most pro bably consisting of subunits of about 70 000 molecular weight.[14] The enzyme is not located in the vacuole of the yeast cell.[67] Activity can be assayed with the synthetic peptide substrate benzyloxycarbonyl-Gly-Gly-Leu-4-nitroanilide in the moderate alkaline pH range of 8 to 8·5. Cleavage occurs at the 4-nitronanilide bond. The enzyme exhibits a small activity against [^{3}H] methylcasein.

Mercurials were found to be strong inhibitors of the enzyme activity.[14]

Other soluble proteinases. In addition to the well characterized proteinases yscD and yscE described above, a variety of additional soluble endopeptidases has been found,[40,71] but these enzymes have not yet been extensively characterized.

As with proteinases yscD and yscE detection of a variety of proteinases was only possible in strains lacking the vacuolar endoproteinases and carboxypeptidases of broad specificity.[40,71] These strains also made possible the detection of three distinct [^{3}H] methylcasein-splitting activities which together comprise less than 1% of the total [^{3}H] methylcasein-splitting activity measurable in wild type cells. One of these activities, called proteinase yscK, can be activated by ATP as well as polyvalent ions.[71]

An endopeptidase of $Mr = 43\,000$ able to cleave the Leu-enkephaline derivative Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Phe-Ala-NH_2 between the Arg-Arg pair at an optimum pH in the neutral range has been reported. Because of its specificity cleaving at pairs of basic amino acid residues, the signals of pro-hormone processing, the enzyme was called pro-pheromone convertase Y. Activity is strongly

inhibited by the serine residue modifying agents diisopropylfluorophosphate and phenylmethylsulphonyl fluoride.[82]

In addition to the vacuolar aminopeptidases yscI and yscCo and the periplasmic aminopeptidase yscII a variety of soluble aminopeptidase activities have been found.[13,69,83,84] Enzyme activity named aminopeptidase yscIII having a molecular weight of either 30 000[13] or 34 000[83] has been described. The different substrate specificity reported, however, may point to two different enzymes. Four leucine aminopeptidases (LAPI to LAPIV) have been described in reference 84. Only one of them (LAPIV) can be identified with certainty with one of the known aminopeptidases, the vacuolar aminopeptidase yscI. LAPI may be identical with aminopeptidase yscII. LAPI and LAPII are metallo-exopeptidases of $M_r = 85\,000$ splitting leucine- and lysine-bound organic chromophores and are subject to EDTA inhibition, LAPIII, a protein of $M_r = 95\,000$ is not inhibited by EDTA.[84] LAPII and LAPIII are most probably among the additional eleven aminopeptidase activities—aminopeptidase yscIV to aminopeptidase yscXIV—that have been detected by Achstetter *et al.*[69] Some of these activities have been characterized to some extent. Aminopeptidase yscV is a sulphydryl exopeptidase of about 170 000 molecular weight, it is sensitive to Hg^{2+} ions and insensitive to nitrilotriacetic acid. Aminopeptidase yscVIII is a metallo exopeptidase of about 165 000 molecular weight insensitive to Hg^{2+} ions, but strongly inhibited by nitrilotriacetic acid. Aminopeptidase yscXI is a sulphydryl metalloexopeptidase of about 110 000 molecular weight, sensitive to both Hg^{2+} ions and nitrilotriacetic acid. Aminopeptidase yscXII is a sulphydryl metallo-exopeptidase of about 97 000 molecular weight and, like aminopeptidase yscXI, sensitive to both Hg^{2+} ions and nitrilotriacetic acid. Aminopeptidase yscXII is present preferentially in exponentially growing cells whereas aminopeptidase yscXI is present in both exponentially growing and stationary phase cells. All these characterized aminopeptidases are sensitive to the actinomycete inhibitor bestatin.[69]

Three additional dipeptidyl aminopeptidases have been described as well, namely dipeptidyl aminopeptidase yscI, dipeptidyl aminopeptidase yscII and dipeptidyl aminopeptidase yscIII.[69]

Dipeptidyl aminopeptidase yscII has been characterized in some detail. It is a metallo-exopeptidase of about 78 000 molecular weight, active at a slightly acidic pH. It cleaves the chromogenic peptide Val-Ala-4-nitroanilide. The enzyme is sensitive to EDTA and Zn^{2+} ions.[69] A dipeptidase of $M_r = 120\,000$—dipeptidase yscI—has been identified.[2,13] Its activity is dependent on metal ions. The enzyme cleaves only dipeptides composed of L-amino acids, Gly-Leu being among the best substrates.[13]

Three additional carboxypeptidases have been detected in a mutant strain lacking the activities of carboxypeptidase yscY and carboxypeptidase yscS. Carboxypeptidase yscγ is a metallo exopeptidase splitting benzyloxycarbonyl-Ser-Phe, carboxypeptidase yscδ is a high molecular weight serine exopeptidase hydrolysing benzyloxycarbonyl-Ala-Phe and carboxypeptidase yscε is a low molecular weight serine exopeptidase splitting benzyloxycarbonyl-Ala-Phe as well.[85]

Proteinases of unknown localization—membrane-bound proteinases

The enzymes described in this section have been identified in the whole membrane fraction of yeast cells (for references see below).

Proteinase yscF. Proteinase yscF, the product of the *KEX2* gene, is a metallo-sulphydryl endopeptidase active in the neutral pH range.[86,87] The enzyme is completely dependent on Ca^{2+}-ions. No other ion can substitute for Ca^{2+}. Low concentrations of Triton X-100 (< 1%) activate the enzyme. Proteinase yscF is made soluble by Triton X-100 (1%) or Zwittergent 3–14 (0·5%). The enzyme has been identified by its ability to cleave synthetic chromogenic peptide substrates at the C-terminal side of a pair of basic amino acid residues: benzyloxycarbonyl-Tyr-Lys-Arg-4-nitroanilide[86] and butoxycarbonyl - Gln - Arg - Arg - 7 - amido-4-methylcoumarin.[87] Chromogenic peptides containing only a pair of basic amino acid residues as total amino acid sequence or typical trypsin substrates containing only one basic amino acid at the cleavage site are not recognized by the enzyme, pointing to very specific requirements for substrate recognition. Dithiothreitol leads to enzyme inactivation.[86] The enzyme is strongly inhibited by the chelating agents EGTA and EDTA, but not 1,10-phenanthroline. Activity is also abolished by the addition of Zn^{2+} and Hg^{2+} ions.[86,87]

Carboxypeptidase yscα. Carboxypeptidase yscα has been found in a mutant strain lacking the two vacuolar carboxypeptidases yscY and yscS.

It is an exopeptidase active at neutral pH with high specificity towards C-terminal basic amino acid residues of synthetic peptides such as benzyloxycarbonyl-Tyr-Lys-Arg and benzyloxycarbonyl-Tyr-Lys.[86]

Aminopeptidase yscP. Aminopeptidase yscP is a metallo-exopeptidase active in the neutral pH range.[69,71] The enzyme can be assayed using the chromogenic substrate Leu-4-nitroanilide. Chelating agents and the actinomycete inhibitor bestatin are potent inhibitors of aminopeptidase yscP.[69]

Dipeptidyl aminopeptidase yscIV. Membrane-bound dipeptidyl aminopeptidase activity had been first described in reference 88. It was later found that this activity was due to two enzymes differing in their sensitivity to heat, the heat-resistant enzyme called dipeptidyl aminopeptidase A and the heat labile enzyme called dipeptidyl aminopeptidase B.[89] The heat labile enzyme was identified as the vacuolar dipeptidyl aminopeptidase yscV[72,73] (see the section on dipeptidyl aminopeptidase yscV). Dipeptidyl aminopeptidase A—for reasons of consistency with the yeast proteinase nomenclature called dipeptidyl aminopeptidase yscIV—is a serine exopeptidase active in the neutral pH range. The activity can be assayed using the chromogenic peptide substrate Ala-Pro-4-nitroanilide. The enzyme is inhibited by phenylmethylsulphonyl fluoride.[69,89] It is remarkably heat stable at 60°C.[89] The enzyme has been reported to be associated with an organelle containing prochitin synthetase, the chitosome.[90]

Other membrane-bound proteinases. In addition to proteinase yscF two membrane-bound metallo-endopeptidases have been found—proteinase yscG and proteinase yscH.[86] Proteinase yscG has been detected by its ability to split the synthetic peptide substrate Cbz-Ala-Ala-Leu-4-nitroanilide at a bond different from the 4-nitroanilide bond. The enzyme is inhibited by EDTA and is sensitive to the treatment with the reducing agent dithiothreitol (T. Achstetter and D. H. Wolf, unpublished observations). In contrast to proteinase yscF Hg^{2+} ions do not inhibit the enzyme. Proteinase yscH can be assayed with the synthetic peptide substrate acetyl-Ala-Ala-Pro-Ala-4-nitroanilide which is cleaved at a bond different from the 4-nitroanilide bond.[86] The enzyme is inhibited by EDTA, but is insensitive to HG^{2+} ions as well as to the treatment with dithiothreitol (T. Achstetter and D. H. Wolf, unpublished observations).

Earlier observations had located two endoproteolytic activities named proteinase M and proteinase P to a membranous fraction in yeast[40]. Later results obtained with rigorously salt-washed membranes could not confirm these findings. Thus, these activities constitute soluble proteins which are contained in the previously obtained membrane fraction and which under conditions of higher ionic strength were completely removed.[71]

Ion exchange chromatography of the salt wash of a membrane fraction containing mostly peripheral and low concentrations of soluble proteins revealed the existence of a unique aminopeptidase activity named aminopeptidase yscXV. The activity is able to cleave only Leu-4-nitroanilide from all aminoacyl 4-nitroanilides tested including Lys-4-nitroanilide, Phe-4-nitroanilide and Ala-4-nitroanilide (T. Achstetter and D. H. Wolf, unpublished results). This enzyme is detectable only in exponentially growing cells, and is active in the neutral pH range.

PROTEOLYSIS AND BIOLOGICAL CONTROL

Intracellular proteolysis might consist of several different types of proteolysis; there might be highly unspecific proteolysis, catalysed by enzymes of very broad specificity, able to degrade nearly every protein to small peptides or even amino acids. Such processes must be restricted to a very limited and secluded space within the cell. The vacuole (lysosome) is the best candidate for the organelle in which such processes could occur. Intracellular proteolysis might furthermore consist of unspecific proteolysis, leading to degradation of proteins, catalysed however by enzymes of very limited specificity. In this case 'signals' to be attached at desired times must render the protein to be proteolysed 'visible' to the proteinase(s). Such processes need not be limited to a certain cellular compartment, they could occur everywhere in the cell. Intracellular proteolysis might in addition be due to very specific proteolysis, where an enzyme of highly restricted specificity recognizes only one protein or a class of proteins. This possibility could be achieved by a very specific sequence only occurring in a few proteins or a class of proteins. Such a type of proteolysis can take place in every cellular compartment, leading to specific activation, modification or inactivation of respective proteins.

Protein degradation is a process which has long been known to occur in growing and stationary

phase yeast cells.[91-93] Since then it became well known that metabolic conditions have a great influence on the protein degradation rate.[91-95] Degradation rates range from 0·5—1% per hour when cells grow on glucose,[94,95] 2% per hour when cells grow on ethanol,[95] 2–3% per hour when cells starve for nitrogen[94,95] to 3% per hour when cells starve for carbon at low phosphate concentrations.[95] Where the degradation properties of proteins in vegetatively growing cells are concerned, two classes can be distinguished: the bulk of the yeast protein being slowly degraded ($t_{1/2} = 160$ h) and a small portion of the protein being degraded rather quickly ($t_{1/2} = 0{\cdot}8$–$2{\cdot}4$ h).[96] It is clear that such data can provide only a more or less 'static', 'over-all' picture on the intracellular regulation of protein levels by proteinases. Such data cannot give answers about the proteins regulated, the regulating proteinases or the mechanisms involved in the regulatory processes.

Several years ago an investigation was started on the specific, irreversible inactivation of a variety of gluconeogenic enzymes upon addition to glucose to yeast cells growing on a non-fermentable carbon source, a process called carbon catabolite inactivation.[97] The enzymes investigated most thoroughly are fructose-1,6-bisphosphatase,[98] cytoplasmic malate dehydrogenase[99] and phosphoenoylpyruvate carboxykinase.[100] Another process, carbon-starvation-induced inactivation of NADP-dependent glutamate dehydrogenase, was studied.[101] The inactivation of these enzymes was thought to be due to proteolysis as the inactivation processes were found to be irreversible[97,101] and the enzyme proteins cross-reacting with specific antibodies disappeared at the same time as enzyme activities.[102-105] At this time only the vacuolar proteinases were known to exist in yeast. Therefore, members of this class of enzymes were assumed to be responsible for the inactivation processes and, especially, the two endoproteinases yscA and yscB were proposed as the main proteolytic regulatory catalysts. This assumption was supported by biochemical studies which showed that the inactivated enzymes *in vivo* were indeed inactivated *in vitro* by one or both of the two vacuolar endoproteinases.[106-108] Similar biochemical studies led to the hypothesis that chitin synthetase, an enzyme essential for the synthesis of the primary septum of the budding yeast cell, is activated by proteinase yscB *in vivo*.[34,38,109] Other *in vitro* studies led to the hypothesis that proteinase yscB might be involved in a process during vacuole biogenesis, namely the activation and maturation of the inactive proform of carboxypeptidase yscY.[61]

The isolation of mutants lacking the vacuolar enzymes proteinase yscA,[110,111] proteinase yscB,[45,111-114] carboxypeptidase yscY[44,85,115] and carboxypeptidase yscS[85] (the mutations are all supposed to be in the structural genes of the enzymes)[85,111,114]. made the elucidation of their intracellular functions possible. It soon appeared that none of these vacuolar peptidases is involved in the specific primary inactivation events of the above mentioned enzymes *in vivo* nor are they involved in the *in vivo* activation and maturation events proposed.[7,85,110,112,113,115-118] It turned out that the vacuolar enzymes have completely different functions. The outcome of these experiments clearly demonstrated the high value of a genetic approach to the question about proteinases and physiological function.

Function of vacuolar proteinases

On the basis of studies using mutant strains lacking proteinase yscA (*pra1*), proteinase yscB (*prb1*), carboxypeptidase yscY (*prc1*) and carboxypeptidase yscS (*cps1*) the major function of these enzymes was found to reside in the differentiation process of sporulation which is triggered by nitrogen starvation.[85,110,112,113,115,119,120] Diploid *MATa*/*MATα* strains, homozygous for the absence of proteinase yscA[110] or yscB[112,113,119] showed a reduced protein degradation rate of 30% and 40–50%, respectively, as compared to diploid *MATa*/*MATα* wild type cells. Sporulation rate in diploid strains lacking one or the other endoproteinase was considerably reduced.[110,112,113]

Diploid *MATa*/*MATα* double mutant strains homozygous for the absence of both endoproteinases yscA and yscB have lost 85% of the protein degradation capacity under sporulation conditions as compared to wild type *MATa*/*MATα* cells and completely cease to sporulate (U. Teichert and D. H. Wolf, unpublished results).

Diploid *MATa*/*MATα* cells devoid of the activities of carboxypeptidase yscY and/or yscS do not show any considerably unimpaired production of spores.[85,115,119] However, diploid *MATa*/*MATα* double mutant strains lacking one of the endoproteinases (proteinase yscB) in addition to carboxypeptidase yscY show only a rate of 10% of sporulation visible under the microscope as compared to wild type diploids.[85,120]

Diploid *MATa*/*MATα* triple mutant strains

lacking the two carboxypeptidases yscY and yscS in addition to proteinase yscB nearly cease to sporulate (sporulation rate <3% as compared to wild type).[85,120] Thus, the vacuolar endoproteinases yscA and yscB and the two vacuolar carboxypeptidases yscY and yscS are necessary enzymes for the differentiation process of sporulation. Here they most likely provide amino acids at the expense of unneeded (vegetative) cell protein for new protein synthesis for the spores to be formed. Earlier biochemical data showed that proteinase yscA and yscB[32,33] as well as carboxypeptidase yscY[33] increase considerably in activity upon shift of diploid cells to sporulation medium. This fits quite well the results concerning the function of the above four peptidases in sporulation obtained by means of genetic analysis.

In vegetative haploid cells, proteinase yscB deficiency causes a decrease in protein degradation of 40–60% as compared to wild type when cells undergo starvation conditions.[112] Furthermore the two carboxypeptidases yscY and yscS are involved in the metabolism of exogenously supplied peptides. Both enzymes enable the cell to grow on benzyloxycarbonyl-Gly-Leu as sole nitrogen source.[12,85] Aminopeptidase yscI has also been implicated in the utilization of peptides.[84]

The vacuolar proteinases are obviously involved in highly non-specific protein and peptide degradation. No other function has been found for the vacuolar proteolytic enzymes so far.

The capacity of cell extracts of a quadruple mutant strain lacking the two endoproteinases yscA and yscB as well as the two carboxypeptidases yscY and yscS to degrade an 'unspecific' substrate such as [^{3}H] methylcasein is reduced by 99% as compared to a wild type strain harbouring these enzymes.[71] Thus, one can assume that these enzymes represent the major unspecific peptidases of the yeast cell. All genetic and biochemical data available on the physiology of the vacuole concerning its proteolytic function support the concept[15] of this organelle being a digestive compartment of the yeast cell.

The broad specificity of the vacuolar proteinases has in many cases been the reason for severe artifacts occurring during enzyme purification and analysis and in the determination of enzyme instability (see for example references 121, 122). The use of strains now available, which lack these highly unspecific proteinases, should greatly facilitate purification and analysis of yeast proteins.

Function of mitochondrial proteinases

The mitochondrial matrix proteinase (proteinase yscMpI) was purified and characterized on the basis of its high specificity for the catalysis of mitochondrial precursor protein processing. The enzyme cleaves precursors *in vitro* to proteins imported into the mitochondrial matrix and the mitochondrial inner membrane.[74–76] The enzyme also appears to be involved in the two-step processing of precursors to proteins destined for the outer face of the inner mitochondrial membrane and for the intermembrane space.[75,123,124] It cleaves these precursors to intermediate forms. The second cleavage, converting the intermediate forms to the mature forms, is mediated by a second proteinase which has not been identified as yet.[123,124] In contrast to the vacuolar proteinases the mitochondrial matrix proteinase (proteinase yscMpI) shows a unique degree of specificity. It cleaves mitochondrial precursors only.[74,75] Cytochrome *c* oxidase subunit V is shown to be correctly processed by the enzyme.[76] Thus, here the biochemical data leave little doubt that this enzyme is really involved in these processes *in vivo*, even though no mutants of the enzyme have been studied as yet.

The situation is completely different with the mitochondrial proteinases of low molecular weight acting on benzoyl-D,L-arginine-β-naphthylamide and benzoyl-D,L-arginine-4-nitroanilide: These enzymes are thought to be involved in the breakdown of mitochondrial translation products.[77] However, until mutants are available to test this hypothesis, this proposal must remain tentative.

Function of periplasmic proteinases

Mutants devoid of aminopeptidase yscII (*ape2*) were isolated. The enzyme is not involved in any process vital for vegetative growth on minimal or complete medium. Neither mating nor the differentiation process of sporulation is disturbed. The enzyme might be involved in utilization of exogenously supplied peptides as nitrogen source (Hirsch, H. H., Suarez Rendueles, M. P., Achstetter, T. and Wolf, D. H., in preparation).

Function of membrane-bound proteinases

Three membrane-bound enzymes—proteinase yscF, dipeptidyl aminopeptidase yscIV and car-

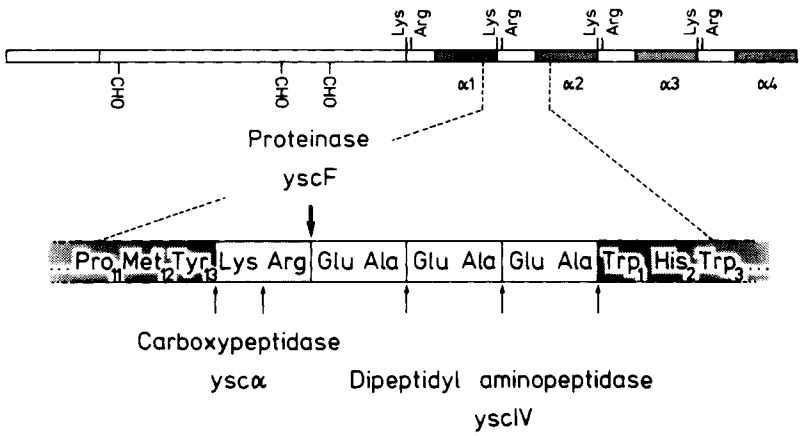

Figure 1. α-Factor pheromone precursor maturation and the enzymes involved. For details see text. (According to reference 86.)

boxypeptidase yscα were shown to be involved in the biosynthesis of the mating pheromone α-factor.[86,87,89] In the yeast *S. cerevisiae* α-factor, one of the two oligopeptide pheromones triggering sexual conjugation of the haploid cell types *MATa* and *MATα*[125,126] is synthesized as a high molecular weight precursor protein which must undergo proteolytic maturation to yield the active pheromone.[127,128] The cloned α-factor precursor gene (*MFα1*) revealed that α-factor is present in four repeats in the precursor molecule. The repeats are flanked by peptide spacers, each starting with the amino acid sequence Lys-Arg and followed by -(Glu-Ala)$_2$-, -(Glu-Ala)$_3$- or -Glu-Ala-Asp-Ala-Glu-Ala- sequences[129] (Fig. 1). How does processing of this molecule proceed? Sequences of two basic amino acids have been found in many mammalian hormone precursor molecules and are thought to represent the initial processing sites.[4] Based on the finding that chromogenic peptide substrates represent an extremely powerful tool to uncover proteinases in yeast,[14,40,71,81] the chromogenic peptide substrate benzyloxycarbonyl-Tyr-Lys-Arg-4-nitroanilide harbouring the carboxyterminal tyrosine residue of the α-factor and the consecutive Lys-Arg-sequence of the spacer peptide was introduced into a search for the processing endoproteinase.[86] In another approach the chromogenic peptide butoxycarbonyl-Gln-Arg-Arg-7-amido-4-methylcoumarin was used.[87] The search resulted in the detection of the Ca^{2+} ion-dependent proteinase yscF[86] or 'lysine-arginine-cleaving endopeptidase'[87] which is strictly specific for splitting these peptides after the Lys-Arg or Arg-Arg bond. Strong evidence that this enzyme is the maturase responsible for the initial cut of the α-factor precursor molecule comes from a mutation (*kex2*)[130] which causes a defect in killer toxin secretion (the phenotype found after mutant selection), and which leads to α-sterility[130,131] and to accumulation of an overglycosylated α-factor precursor.[132] Proteinase yscF activity is absent in this mutant.[86,87] Cloning of the *KEX2* gene restores mating ability of *MATα kex2* mutants and shows this gene to be the structural gene of the enzyme.[87]

Another maturase, called pro-pheromone convertase Y, has been proposed to be the catalyst of this initial processing step.[82] However, no data relevant for the processing *in vivo* were presented, leaving severe doubts on the correctness of this hypothesis.

Splitting of the α-factor precursor molecule after the Lys-Arg amino acid pair leaves this amino acid sequence carboxyterminally connected to the α-factor molecules and -(Glu-Ala)$_2$-, -(Glu-Ala)$_3$- or Glu-Ala-Asp-Ala-Glu-Ala sequences connected to the amino terminus of the pheromone (Fig. 1). Carboxypeptidase yscα is proposed to be responsible for further maturation at the carboxyterminal cleavage site of α-factor. The enzyme is highly specific for the carboxyterminal cleavage of basic amino acid residues from synthetic sequences which mimic the cleavage site of the α-factor precursor molecules.[86] The aminoterminal extensions remaining on α-factor after the initial cut by

Table 1. Proteinases of the yeast *S. cerevisiae*

Enzymes	Structural gene(s)	Characteristics	Cellular localization	Cellular role(s)	Previous nomenclature
Proteinase yscA	*PRA1*	carboxylic endopeptidase	vacuole (soluble)	protein degradation nitrogen metabolism	proteinase A
Proteinase yscB	*PRB1*	serine sulphydryl endopeptidase	vacuole (soluble)	protein degradation nitrogen metabolism	proteinase B
Proteinase yscD	unknown	metallo (sulphydryl) endopeptidase	non-vacuolar (soluble)	unknown	
Proteinase yscE	unknown	sulphydryl endopeptidase	non-vacuolar (soluble)	unknown	
Proteinase yscF	*KEX2*	metallo sulphydryl endopeptidase (Ca^{2+})	membrane fraction (Golgi?)	precursor processing α-factor, killer factor	endoproteinase Lys-Arg
Proteinase yscG	unknown	metallo endoproteinase	membrane fraction	unknown	—
Proteinase yscH	unknown	metallo endopeptidase	membrane fraction	unknown	—
Proteinase yscK	unknown	unknown	unknown (soluble)	unknown	—
Proteinase yscMpI	unknown	metallo endoproteinase (Zn^{2+})	mitochondrial matrix (soluble)	precursor processing of nuclear coded mitochondrial proteins	matrix located proteinase
3 mitochondrial proteinases	unknown	serine sulphydryl endopeptidases	inner mitochondrial membrane	unknown	—
Propheromone convertase Y	unknown	serine endopeptidase	unknown (soluble)	unknown	—
Proteinases yscM, yscP and an additional number of unknown proteinases					—
Aminopeptidase yscI	*LAP4?*	metallo sulphydryl exopeptidase (Zn^{2+})	vacuole (soluble)	unknown	aminopeptidase I, V, aminopolypeptidase. LAPIV, aminopeptidase III
Aminopeptidase yscII	*APE2 (LAP1?)*	metallo sulphydryl exopeptidase (Zn^{2+})	periplasm (soluble)	unknown	aminopeptidase II, (LAPI?)
Aminopeptidase yscIII	unknown	unknown	unknown (soluble)	unknown	aminopeptidase III
11 aminopeptidases (including probably LAPII and LAPIII): aminopeptidases yscIV-XIV	unknown	exopeptidases	unknown (soluble)	unknown	aminopeptidases IV-XIV

Table 1. *Continued*

Enzymes	Structural gene(s)	Characteristics	Cellular localization	Cellular role(s)	Previous nomenclature
Aminopeptidase yscCo	unknown	metallo sulphydryl exopeptidase (Co^{2+})	vacuole (soluble)	unknown	aminopeptidase Co
Aminopeptidase yscP	unknown	metallo exopeptidase	membrane fraction	unknown	aminopeptidase P
Aminopeptidase yscXV	unknown	unknown	membrane fraction	unknown	—
Dipeptidyl aminopeptidase yscI	unknown	unknown	unknown (soluble)	unknown	dipeptidyl aminopeptidase I
Dipeptidyl aminopeptidase yscII	unknown	metallo exopeptidase	unknown (soluble)	unknown	dipeptidyl aminopeptidase II
Dipeptidyl aminopeptidase yscIII	unknown	unknown	unknown (soluble)	unknown	
Dipeptidyl aminopeptidase yscIV	*STE13*	serine exopeptidase	membrane fraction (Golgi?)	precursor processing α-factor	dipeptidyl aminopeptidase A, X-prolyldipeptidyl aminopeptidase
Dipeptidyl aminopeptidase yscV	unknown	serine exopeptidase	vacuole (membrane)	unknown	dipeptidyl aminopeptidase B, X-prolyldipeptidyl aminopeptidase
Carboxypeptidase yscY	*PRC1*	serine sulphydryl exopeptidase	vacuole (soluble)	protein degradation, nitrogen metabolism	proteinase C, carboxypeptidase Y
Carboxypeptidase yscS	*CPS1*	metallo exopeptidase (Zn^{2+})	vacuole (soluble)	protein degradation nitrogen metabolism	carboxypeptidase S
Carboxypeptidase yscα	unknown	unknown	membrane fraction	processing of α-factor precursor?	—
Carboxypeptidase yscγ	unknown	metallo exopeptidase	unknown (soluble)	unknown	carboxypeptidase γ
Carboxypeptidase yscδ	unknown	serine exopeptidase	unknown (soluble)	unknown	carboxypeptidase δ
Carboxypeptidase yscε	unknown	serine exopeptidase	unknown (soluble)	unknown	carboxypeptidase ε
Dipeptidase yscI	unknown	metallo exopeptidase (Mg^{2+})	unknown (soluble)	unknown	dipeptidase

For detailed characteristics of the proteinases and for references see text.

proteinase yscF are removed by dipeptidyl aminopeptidase yscIV (previously called dipeptidyl aminopeptidase A)[89] (Fig. 1). According to the specificity of the enzyme, removal of amino acids occurs pairwise as has been found in the maturation process of the bee venom melittin.[133] Strong evidence that dipeptidyl aminopeptidase yscIV (dipeptidyl aminopeptidase A) is responsible for this maturation step comes from a mutant lacking *STE13* gene product activity. The *STE13* gene is required for fertility of *MATα* cells but not of *MATa* cells.[134] *MATa* mutant cells defective in this gene secrete biologically inactive α-factor molecules harbouring part of the aminoterminal extension remaining after the initial cut of the precursor by proteinase yscF and they lack dipeptidyl aminopeptidase yscIV activity. Cloning of the *STE13* gene in *MATα ste13* mutants leads to an overproduction of dipeptidyl aminopeptidase yscIV activity and cures sterility.[89] The scheme of α-factor processing is depicted in Figure 1.

More than 40 proteolytic enzymes have been detected to date in *S. cerevisiae* (see Table I). The function of nine of them could be unequivocally uncovered up to now. In addition, however, a variety of processes of proteolytic nature have been found in yeast for which the catalyst has yet to be detected.

PROTEOLYTIC PROCESSES, FOR WHICH THE PROTEINASES HAVE TO BE FOUND

Catabolite inactivation

Addition of glucose to cells growing on a non-fermentable carbon source leads to specific inactivation of a very limited set of enzymes, a process called catabolite inactivation (for a review see reference 97). The enzymes analysed best and for which disappearance of the protein has clearly been shown are the gluconeogenic enzymes fructose-1,6-bisphosphatase,[98,102] cytoplasmic malate dehydrogenase,[99,103,135] and phosphoenolpyruvate carboxykinase.[100,136,137] Also for the maltose and galactose uptake systems,[138-140] uridine nucleosidase[141] and aminopeptidase yscI[142] glucose-triggered inactivation has been reported. As outlined in the section on proteolysis and biological control, involvement of vacuolar proteinases in the primary inactivation events of these enzymes, proposed on the basis of biochemical data, has been ruled out on the basis of genetic studies (for a review see reference 7). The catalyst(s) of these events have to be found.

In the case of fructose-1,6-bisphosphatase it was recently shown that cAMP-dependent phosphorylation leads to inactivation of the enzyme and precedes disappearance of its protein moiety.[143-146] It has yet to be shown whether vacuolar proteinases or enzymes localized in other cellular compartments are responsible for the degradation of the inactivated fructose-1,6-bisphosphatase.

Starvation initiated inactivation

NADP-dependent glutamate dehydrogenase is inactivated during carbon starvation of cells paralleled by disappearance of its immunologically detectable protein.[101,105] The vacuolar endoproteinases yscA and yscB are not involved in this process:[110,112,117] the catalyst has yet to be found. The catalysts for the degradation of the phosphorylated NAD-dependent glutamate dehydrogenase generated under carbon starvation of cells has to be discovered in addition.[147]

Proteolytic events during protein synthesis and secretion

Eukaryotic, cytoplasmic protein synthesis is initiated by methionine. This N-terminal methionine is removed from proteins when alanine, serine; glycine, proline, threonine and valine is the penultimate amino acid. Excision does not occur when methionine precedes residues of arginine, asparagine, aspartic acid, glutamine, glutamic acid, isoleucine, leucine, lysine or methionine.[148] The aminopeptidase responsible has yet to be identified. The enzyme might well be among the many aminopeptidases described (see Table 1).

The secretory pathway in yeast has been well defined by mutants temperature sensitive for growth and the export of secretory proteins (*sec*).[149,151] Based on studies with these mutants the secretory pathway has been defined as leading from:

(a) endoplasmic reticulum → Golgi → vesicles → secretion

(b) endoplasmic reticulum → Golgi → vacuoles.

The mutants allow a temperature-sensitive blockage of the delivery of secretory proteins either into the endoplasmic reticulum, from the endoplasmic reticulum to the Golgi complex, from the Golgi complex to secretory vesicles (or—for vacuolar enzymes—from the Golgi complex to the vacuole), and from the secretory vesicles to the external space. By these means, the mutants cause

the accumulation of secretory proteins in the respective cell compartment from which delivery is blocked and allow analysis of the molecular forms of the secretory proteins retained in this compartment. Studies on these mutants made the detection of the existence of α-factor as a high molecular weight precursor possible and allowed tracing of its proteolytic processing along the secretory pathway.[127,128]

Invertase, an enzyme encoded by the *SUC2* gene and required for growth of yeast cells on sucrose as sole carbon source, is secreted via the yeast secretory pathway.[151] The enzyme bears a signal sequence characteristic for secretory proteins.[152,153] Comparison of the molecular weight of the enzyme protein as deduced from the DNA sequence with the protein moiety accumulating in *sec18* cells blocked in the delivery step from the endoplasmic reticulum to the Golgi as well as with the protein moiety of external invertase shows that the signal sequence is removed upon transfer of the protein into the endoplasmic reticulum.[154] Also acid phosphatase, which is secreted to the cell surface, contains a signal peptide which is cleaved off during the secretion process.[155] The catalyst of these processes, signal peptidase, should be located in the endoplasmic reticulum. The enzyme has yet to be found.

As shown with carboxypeptidase yscY, transport of vacuolar enzymes also follows part of the secretory pathway. Sorting from secreted proteins occurs in the Golgi.[156] Carboxypeptidase yscY is synthesized as a precursor of higher molecular weight containing an amino-terminal peptide extension.[61,156,157] Proteolytic cleavage of the 67 000 to 69 000 molecular weight precursor to the 61 000 molecular weight mature form[61] occurs either in transit from the Golgi body or after the proenzyme arrives in the vacuole.[156] Also the two vacuolar endoproteinases, proteinase yscA and proteinase yscB, were found to be synthesized as higher molecular weight precursor molecules of $M_r = 52\,000$ and $M_r = 42\,000$, respectively.[23] These precursors are processed to their mature forms of $M_r = 42\,000$ for proteinase yscA and $M_r = 33{,}000$ for proteinase yscB.[23] Zubenko *et al.*[158] also reported processing of higher molecular weight precursor molecules of proteinase yscA to the mature enzyme. However, these results do not agree with those reported in reference 23. Zubenko *et al.*[158] report a molecular weight of 58 000 for mature proteinase yscA as compared to $M_r = 41\,500$[21] or $M_r = 45\,000$[22] published for the highly purified protein and found by Mechler *et al.*[23] Proteinase yscA was shown to contain 8–8·5% carbohydrate.[21,22] Mechler *et al.*[23] found precursor and mature form of proteinase yscA bearing two asparagine-linked carbohydrate chains, sensitive to removal with endoglycosidase H, whereas Zubenko *et al.*[158] do not find any carbohydrate sensitive to endoglycosidase H. The reason for the discrepancy of these results is as yet unknown.

The proteolytic enzyme responsible for maturation of the precursor proteins of the vacuolar proteinases has yet to be found. Mutants with defects in the *PEP4* gene have been isolated and shown to be defective for a variety of vacuolar hydrolase activities.[44,118,159] Using immunochemical techniques it was shown that the *pep4* mutant accumulates precursor molecules of carboxypeptidase yscY[118] and proteinase yscB.[111] No mature enzyme forms can be detected.[111–118] The pleiotropic nature of the *pep4* mutation suggests that the processing machinery—probably the proteinase itself—is disturbed by the defect.

Similar to the mating pheromone of *MATα* cells, the mating pheromone secreted by *MATa* cells—a-factor—is a small peptide. It contains a sequence of 11 amino acids.[125] A DNA stretch has been found in *MATa* cells, which could potentially encode an a-factor precursor polypeptide of 36 amino acids containing a single copy of a-factor.[160] The a-factor sequence is preceded by a N-terminal peptide extension containing a pair of basic amino acid residues (Lys-Lys) which may provide the signal for the initial proteolytic processing step[160] as is the case for α-factor. Proteinase yscF, the α-factor precursor maturase[86,87] does not seem to be responsible for this initial processing step of the a-factor precursor as *MATa* cells of mutants defective in the structural gene for proteinase yscF87—*KEX2*—are not serile.[130] The catalysts for liberation of mature a-factor from the precursor have yet to be found.

S. cerevisiae killer strains secrete a polypeptide toxin which kills sensitive cells of their own species and frequently those of other species and genera of yeast. The killer determinant is a cytoplasmically inherited double-stranded RNA (the M double-stranded RNA) which encodes a toxin precursor (for reviews see references 131, 161). Secretion of the toxin occurs via the normal secretory pathway.[162] The protoxin is a glycosylated protein of $M_r = 42$–$44\,000$ consisting of a protein portion of $M_r = 34$–$35\,000$.[163] A minimum of three cleavage events must occur to yield the mature toxin

($M_r = 18\,500$) composed of two polypeptide chains, α and β,[164] The proposed cleavage sites of the toxin precursor with its domains δ-α-γ-β are an Arg-Glu bond between the δ-domain and the α-subunit of the toxin, a Trp-Gly bond between the α-subunit and the γ-domain and an Arg-Tyr bond (the Arg is part of a basic amino acid pair of the sequence *Lys-Arg*-Tyr) between the γ-domain and the β-subunit of the toxin.[161] Processing of the precursor to killer toxin is abnormal in *kex2* mutants.[131,161,162] *Kex2* mutants lack proteinase yscF, an enzyme specific for cleavage after pairs of basic amino acid residues and responsible for maturation of the mating pheromone α-factor.[86,87] From its high specificity for cleaving after pairs of basic amino acid residues but its broad specificity for the amino acid following the basic amino acid pair[86,87] one might predict that proteinase yscF is responsible for cleavage of the Arg-Tyr bond in the precursor between the γ-domain and the β-subunit of the toxin. By this split the β-subunit of the toxin becomes liberated. Catalysts of the other processing steps have yet to be identified. Whether the *KEX1* gene[165] codes for one of the other processing proteinases has to be elucidated.

Chitin has been found to be the constituent of the primary septum that forms between mother and daughter cell during the budding process of vegetatively growing yeast cells.[109,166] The synthesis of chitin is catalysed in a strongly time- and location-dependent process.[109] The catalyst is chitin synthetase, an enzyme present in zymogen form in the plasma membrane of the yeast cell.[109, 167] It had been proposed that one of the vacuolar proteinases—proteinase yscB—is responsible for the activation process,[34,38,109] however, this proposal was ruled out on the basis of genetic studies (see the section on proteolysis and biological control and for a review see reference 7). Whether the activity described recently[168] is actually responsible for chitin synthetase activation rests on future studies. As the synthesis of chitin must be strongly controlled with time a programmed inactivation of chitin synthetase must be hypothesized.

Pheromone inactivation

Hormones (pheromones) are signals triggering the induction of cellular metabolic changes. As such they must have a strictly limited life span. The mating pheromone α-factor is inactivated by *MATa* cells. *MATa* cells have been shown to cleave the tridecapeptide α-factor between Leu_6 and Lys_7 of the sequence. An enzyme capable of cleaving α-factor at the sequence indicated is associated with the membrane.[169] *sst1 (bar1)* mutants are supersensitive to α-factor and are deficient in an activity inactivating α-factor.[170-172] It is not clear, however, whether *SST1* (*BAR1*) actually encodes an α-factor inactivating peptidase and whether this enzyme has anything to do with the peptidase splitting at the Leu_6-Lys_7 position of the pheromone.

Proteolysis in mutagenesis and repair

In *Escherichia coli* DNA damage leads to a pleiotropic SOS response which is due to DNA repair, prophage induction, filamentation and to the induction of a proteinase, the *rec*A protein, which is believed to be essential for the regulation of SOS functions.[173, 174] It is speculated that proteolytic activities may act upon different repressors giving rise to the pleiotropic SOS response.[173-176] Also in eukaryotes the involvement of proteolytic processes in epigenetic and genetic events triggered by radiation or chemical carcinogenesis has been indicated.[177] In *S. cerevisiae*, of the five proteinases measured (proteinase yscA, proteinase yscB, carboxypeptidase yscY, aminopeptidase yscI and aminopeptidase yscII), the vacuolar proteinase yscB showed a significant increase in u.v. irradiated cells.[178] The proposal was made that this increase in the proteinase yscB level may be related to the triggering of the mutagenic DNA repair.[178,179] It is completely unknown, however, in which way the enzyme should participate in this repair process. Nothing is known about a probable involvement of more specific proteinases in the induction of mutagenic DNA repair.

Degradation of aberrant proteins

The cell has to take care that aberrant and nonfunctional protein is degraded rather quickly to avoid accumulation of protein waste and disturbance of cell metabolism. Aberrant proteins and protein fragments due to mutation have been found to be greatly reduced in cells.[180-182] A defect in processing of the precursor to the 25S RNA of the 60S subunit of the ribosome due to mutation leads to rapid degradation of the unassembled proteins of the 60S ribosomal subunit.[183] Mutations in either one of the two *FAS* genes which code for the two subunits of the fatty acid synthetase complex lead to absence of immunologically detectable fatty acid synthetase complex.[184,185] One possible explanation for this

phenotype is the rapid degradation of aberrant and unassembled subunits. Also unassembled mitochondrial proteins are thought to be degraded.[186] A mutationally altered carboxypeptidase yscY protein was shown to be rapidly degraded.[111] Mutants defective in the vacuolar endoproteinases yscA and yscB as well as in carboxypeptidase yscS revealed that these enzymes are not involved in the degradation process of the carboxypeptidase yscY mutant protein (Mechler, B and Wolf, D. H., in preparation). This clearly indicates that yeast must contain additional degradative potential besides the known vacuolar proteinases. Ubiquitin, a polypeptide of 8500 molecular weight, has been found in yeast[187] and among other proteolytic systems, yeast may contain the ATP- and ubiquitin-dependent degradative system found in several eukaryotic cell types.[5,188–190] An energy-requiring process leads to conjugation of ubiquitin to proteins which are subsequently degraded.[5,188]

The ubiquitin gene of yeast has been cloned and sequenced.[191] From the DNA sequence it could be deduced that ubiquitin is synthesized as a polyprotein containing six ubiquitin molecules bound in a head-to-tail arrangement. The ubiquitin molecules are joined directly via Gly-Met peptide bonds between the last and the first residue of mature ubiquitin, respectively.[191] A processing proteinase splitting this Gly-Met bond and by this liberating mature ubiquitin from the precursor has to be postulated.

CONCLUDING REMARKS

More than 40 proteolytic enzymes have been described up to now in yeast. Some of these enzymes are well characterised, others have just been identified on the basis of a few parameters. Research in the last ten years has shown that unequivocal assignment of a certain proteinase to a certain intracellular process is nearly possible only by virtue of a combination of genetic and biochemical tools. Here yeast has its great advantage over other eukaryotic systems—it is easily accessible to genetics and molecular biology.

For many proteolytic processes in yeast we are still without knowledge of the catalysing proteinase. We must suspect that some of these processes are catalysed by as yet unknown proteolytic enzymes. Thus, the list of proteinases summarized in Table I will most likely increase even more. On the other hand, there is no doubt that the proteolytic processes known at present in yeast represent only the tip of an iceberg. We can therefore look forward to exciting discoveries in a field of research that, in the eyes of many earlier researchers, seemed to deal with boring catalysts present in the cell only to annoy biochemists who wanted to purify proteins.

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