

Hormone (pheromone) processing enzymes in yeast

The carboxy-terminal processing enzyme of the mating pheromone α -factor, carboxypeptidase *ysc α* , is absent in α -factor maturation-defective *kex1* mutant cells

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Carboxy-terminal processing of the mating pheromone α -factor of the yeast *Saccharomyces cerevisiae* has been assumed to be due to the action of carboxypeptidase *ysc α* [(1985) EMBO J. 4, 173–177]. Here it is shown that a mutant (*kex1*) defective in α -factor maturation is defective in carboxypeptidase *ysc α* activity, indicating that the enzyme is indeed the processing catalyst. It is proposed that carboxypeptidase *ysc α* is the product of the *KEX1* gene.

Sex pheromone; Protein precursor; Hormone processing; Carboxypeptidase *ysc α* ;
(Yeast mutant, *Saccharomyces cerevisiae*)

1. INTRODUCTION

Yeast cells can exist in two haploid cell types of different sex, called *a* and α , which can mate to form *a*/ α diploids. This mating event is triggered by two oligopeptide hormones (pheromones) called *a*- and α -factor, whereby *a*-factor is secreted by *a*-cells and acts on cells of the α -mating type and α -factor is secreted by α -cells and acts on cells of the *a*-mating type [1,2]. The secreted, biologically active α -factor pheromone is a tridecapeptide which is intracellularly synthesized as a precursor protein [3,4].

The sequenced gene of the α -factor precursor uncovered the possible processing sites of the precursor protein to yield the active pheromone [5] (fig.1). Four repeats of the α -factor are flanked by peptide spacers which start with a Lys-Arg se-

quence each and are followed by Glu-Ala or Asp-Ala sequences [5]. As proposed for maturation of mammalian peptide hormones [6], for yeast α -factor it could be shown that the initial processing event is started by a highly specific endoproteinase called proteinase *yscF* or *KEX2* endoprotease which splits after the basic amino acid pair Lys-Arg [7,8]. The cleavage step brought about by the above membrane-bound enzyme leaves α -factor molecules which need further processing at the amino- and carboxy-termini (fig.1). Amino-terminal processing of the four liberated α -factor molecules is exerted by a membrane-bound dipeptidylaminopeptidase called dipeptidylaminopeptidase A or dipeptidylaminopeptidase *yscIV*, which removes Glu-Ala and Asp-Ala dipeptides (fig.1) ([9], reviews [10–12]). The conclusions about the involvement of proteinase *yscF* and dipeptidylaminopeptidase *yscIV* in the α -factor pheromone maturation event were strongly based on studies with mutants defective in the above enzymes [7–9]. Carboxy-terminal processing is necessary for three

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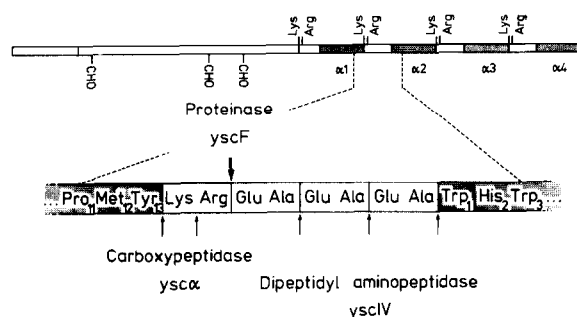


Fig.1. Maturation of α -factor and the peptidases involved (according to [7]).

of the four α -factor molecules (see fig.1). We have previously detected a membrane-bound carboxypeptidase activity, called carboxypeptidase yscA, which specifically cleaves the basic amino acids Lys and Arg from a model peptide which resembles the carboxy-terminus of α -factor and the penultimate spacer region (Cbz-Tyr-Lys-Arg) [7]. We were able to show that carboxypeptidase yscA has characteristics of the vacuolar carboxypeptidase yscY. The enzyme is most active in the slightly acidic and neutral pH range and is completely inhibited by phenylmethylsulfonyl fluoride and low concentrations of mercury compounds [13]. The inhibition spectrum indicates carboxypeptidase yscA to be a serine peptidase with a sensitive SH group [13]. No mutants to prove finally the involvement of carboxypeptidase yscA in the α -factor maturation event were known. Recently it was shown that a mutant (*kex1*), defective in killer factor expression [14,15], is defective in killer factor maturation [12,16,17] and produces a significantly reduced amount of mature α -factor [12,17]. Crude α -factor preparations of the *kex1* mutant strain could be activated in vitro with carboxypeptidase B [12,17]. It was furthermore shown that the *KEX1* gene predicts a membrane-bound protein which bears a striking homology with carboxypeptidase yscY [18]. Here we show that carboxypeptidase yscA is lacking in *kex1* mutant cells, indicating that *KEX1* is the structural gene of the enzyme and implying that carboxypeptidase yscA is indeed involved in carboxy-terminal processing of α -factor.

2. MATERIALS AND METHODS

Strain 96 (*a kex1 ade2 thr1*) was obtained from the Yeast Genetic Stock Center, Berkeley. Strains

supersensitive to mating pheromones (RC757 (*α sst2-1 his6 met1 can1 cyh2 rme*), and RC629 (*α sst1-2 ade2-1 ural his6 met1 can1 cyh2 rme*) [20,21]) were a generous gift from Dr R.K. Chan, Cambridge, USA. Strain 96 was crossed with strain BYS232-31-42 (*α prb1-1 prc1-1 cps1-3 lys2 leu2 his7*) [7,19], diploids were isolated, sporulated and tetrads were dissected using standard genetic techniques [22,23]. Spores were tested for mating pheromone secretion on lawns of either strain RC757 (test for α -factor) or strain RC629 (test for α -factor) according to [20,21,24]. Cells were grown for 24 h at 30°C on media containing yeast extract (1%), peptone (2%) and glucose (2%) (YPD). When solid media were used 2% agar was added. Membranes of strains were prepared as outlined in [7]. Carboxypeptidase yscA was tested in the purified membrane fraction of cells at pH 7.2 using the peptide substrate Cbz-Tyr-Lys-Arg or Cbz-Tyr-Lys and carboxypeptidase yscI was measured at pH 7.2 with Cbz-Phe-Leu. Tests were performed at 30°C. The increase in fluorescence brought about by carboxy-terminal liberation of amino acids from the peptide substrates after 5 h reaction time followed by subsequent reaction of the amino acids with *o*-phthalaldehyde was determined as outlined in [7]. The final test volume was 0.4 ml. Aliquots of 20 μ l were removed, diluted into 0.4 ml of 10 mM potassium phosphate buffer, pH 7.3, and 0.2 ml of the fluorescence solution [7] was added. Protein was determined according to [25] using bovine serum albumin as standard. Peptide substrates were from Bachem (Bubendorf, Switzerland).

3. RESULTS AND DISCUSSION

When purified membranes of the *kex1* mutant strain 96 were measured for carboxypeptidase yscA with either Cbz-Tyr-Lys-Arg or Cbz-Tyr-Lys as substrates no activity of the enzyme could be detected. In contrast, membranes of the wild-type strain BYS232-31-42 exhibited a specific activity of carboxypeptidase yscA of 1.8 mU/mg for Cbz-Tyr-Lys-Arg cleavage (table 1) and of 1.7 mU/mg for Cbz-Tyr-Lys hydrolysis. Carboxypeptidase yscA, another membrane-associated carboxypeptidase [13], was not affected in *kex1* mutant cells: an activity of 1.5 mU/mg, similar to that found in wild type (1.2 mU/mg), was measured in strain 96.

Table 1

Specific activity of carboxypeptidase *ysc α* in wild-type and *kex1* mutant cells derived from several tetrads of the cross of strains 96 (*a kex1 ade2 thr1*) \times BYS232-31-42 (*α prb1-1 prc1-1 cps1-3 lys2 leu2 his7*)

Strain	Genotype	Spec. act. (mU/mg)
BYS232-31-42	<i>KEX1</i>	1.8
96	<i>kex1</i>	<0.01
6A	<i>KEX1</i>	1.9
6D	<i>kex1</i>	<0.01
16B	<i>KEX1</i>	2.1
16D	<i>kex1</i>	<0.01
26B	<i>KEX1</i>	1.8
26C	<i>KEX1</i>	1.8
27B	<i>kex1</i>	<0.01
27C	<i>kex1</i>	<0.01
28A	<i>kex1</i>	<0.01
28D	<i>kex1</i>	<0.01
29A	<i>KEX1</i>	1.8
29C	<i>KEX1</i>	1.8

Cells were grown, membranes of strains were prepared and testing of carboxypeptidase *ysc α* activity using Cbz-Tyr-Lys-Arg as substrate were performed as outlined in section 2. Between 33 and 460 μ g protein were included in the test

This finding clearly distinguishes carboxypeptidase *ysc α* and carboxypeptidase *ysc λ* as being two different enzymes of very different substrate specificity. We crossed the *kex1* mutant strain 96 into strain BYS232-31-42 carrying the wild-type *KEX1* allele, isolated diploid *kex1/KEX1* cells, sporulated the heterozygous diploids, dissected tetrads and tested them for their ability to secrete *a*- or α -factor using pheromone-supersensitive tester strains [20,21,24]. As expected from traits coded for by single nuclear genes, from all tetrads analyzed two spores of each tetrad secreted *a*-factor, whereas the two other spores secreted α -factor (not shown). When the α -factor-secreting colonies were analyzed in more detail, two types of colonies could be distinguished: those inhibiting growth of the tester strain to a large extent and thus producing a large halo around themselves (colonies 6A, 26B, 26C) and those which inhibited growth of the tester strain to a lesser extent and therefore producing only a small halo (colonies

6D, 27B, 27C) (fig.2). This phenotypic behaviour is understandable: while wild-type colonies of the α -mating type are able to process the α -factor precursor molecule completely and hence produce four active α -factor molecules from one precursor molecule, *kex1* mutant colonies of the α -mating type should only be able to produce one mature α -factor molecule [12,17]. Only the carboxy-terminal α -factor molecule of the precursor does not need carboxy-terminal processing, which is defective in *kex1* mutant cells, while the others should remain as only partly matured pheromone molecules [12,17] (fig.1). To test whether the carboxypeptidase *ysc α* lesion actually cosegregates with the *kex1* mutation, we analyzed a variety of *kex1* mutant and *KEX1* wild-type cells of the α -mating type of the tetrads isolated for carboxypeptidase *ysc α* activity. As shown in table 1, all mutant colonies exhibiting the *kex1* phenotype are devoid of carboxypeptidase *ysc α* activity, whereas the *KEX1* wild-type cells show normal activity of the enzyme. This result strongly indicates that the 80 kDa carboxypeptidase *yscY*-like enzyme deduced from the nucleotide sequence of the *KEX1* gene [18] is the enzyme, characterized biochemically [13] as a serine-thiol peptidase, carboxypeptidase *ysc α* . Thus, these studies provide proof that the enzyme predicted as being involved in the carboxy-terminal processing of α -factor on the basis of substrate specificity using a peptide which resembles part of

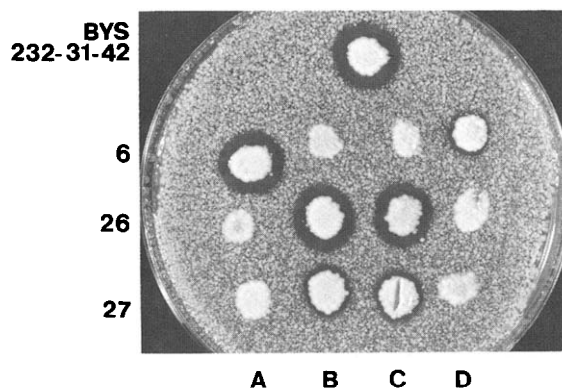


Fig.2. α -Factor production of spores derived from tetrads of a cross of strain 96 (*a kex1 ade2 thr1*) \times BYS232-31-42 (*α prb1-1 prc1-1 cps1-3 lys2 leu2 his7*). The four spores of each tetrad were tested for α -factor production according to [20,21,24] using strain RC629, supersensitive to α -factor action.

the α -factor precursor sequence [7], carboxypeptidase *ysc α* , is indeed the pheromone-maturing catalyst.

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