LOSS OF SPORULATION ABILITY IN A YEAST MUTANT WITH LOW PROTEINASE A LEVELS

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Received 29 January 1979

1. Introduction

Intracellular proteolytic enzymes are assumed to play key roles in the regulation of vegetative growth and of differentiation, i.e., sporulation, of *Saccharomyces cerevisiae* [1–7]. Up to now, two endoproteinases (A, B), two carboxypeptidases (S, Y) and three aminopeptidases have been identified in this organism (reviewed in [2,7]). Despite the recent isolation of mutants lacking carboxypeptidase Y or proteinase B activity, however, so far no abnormalities in the growth or sporulation behaviour of proteinase-deficient yeast cells have been described [8,9]. Some properties of a mutant with low proteinase A activity and defective in sporulation are reported here.

2. Materials and methods

Saccharomyces cerevisiae strain A 364A (*a* ade_1 ade_2 ura_1 tyr_1 his_7 lys_2 gal_1) the temperature-sensitive mutant 17017 (*cdc* 15) derived from A 364A and several segregants from crosses of this mutant with standard yeast strains were kindly provided by Dr L. H. Hartwell, University of Washington, Seattle [10–12]. The cells were routinely grown at 23°C on YM-1 medium as in [12]. In sporulation studies, the methods in [3] were used except that all media were supplemented with 0.01 g/l adenine and uracil, each, and that growth and sporulation were carried out at 23°C. The preparation of crude extracts, the assay of proteinase A activities and all other methods were as in [3].

3. Results

In pioneering studies on the cell division cycle of *Saccharomyces cerevisiae*, Hartwell isolated a great number of temperature-sensitive socalled cdc-mutants which at the restrictive temperature arrest at different steps of the cell cycle [10]. When, because of the suggested trigger function of proteinase B in yeast budding [1], several of these mutants were screened for their proteinase activities, one of them, 17017, was found to exhibit ~5-fold lower specific activity of proteinase A than the parent strain A 364A (table 1). By contrast, the specific activities of proteinase B, carboxypeptidase Y and the proteinase B inhibitor (13, 14) differed by <2-fold in the same early stationary phase cells (data not shown).

Table 1
Proteinase A specific activities in crude extracts from A 364A and 17017

Strain	Proteinase A (U/mg protein)	
	23°C	37°C
A 364A	1.75	1.30
17017	0.36	0.25

The cells were grown in YM-1 medium at 23°C for 36 h. Then 50% of the cultures was collected and assayed for proteinase A activity. The remaining cells were shifted to 37°C for 3.5 h before harvesting

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Incubation of the crude extracts at pH 5.0 and 25°C for 24 h, a condition known to destroy the yeast's own proteinase A inhibitor [15], resulted in 15–40% higher proteinase A activities in both mutant and parent strain extracts, but did not significantly alter the activity ratio between these two enzyme sources. Therefore the low proteinase A activity in 17017 cannot be explained by increased levels of proteinase A inhibitor. As demonstrated in table 1, the proteinase deficiency of 17017 was found not only after a shift of cells to the restrictive temperature of 37°C, but also at 23°C, suggesting that in addition to the temperature-sensitive lesion a second independent mutation may be present. Tetrad analysis of crosses of the mutant with wild-type strains indeed showed temperature sensitivity and low proteinase A activity both segregating in a 2:2 relationship, but rather independently one from the other. Because of the drastic mutagenisation procedure originally utilised for the isolation of the *cdc* mutants, the occurrence of a second independent nuclear mutation in 17017 seemed, however, not very surprising.

When proteinase A was partially purified from both A 364A and 17017 as in [13], no differences in heat stability, relative activity against acid-denatured hemoglobin [15], casein [15], tryptophan synthase [13] or radiolabelled yeast protein [3] and in sensitivity to pepstatin [15] or purified proteinase A inhibitor from yeast [16] could be detected between the two enzyme preparations (H.B., unpublished). This leads me to postulate that the proteinase A deficiency in 17017 is the result of a regulatory mutation which prevents derepression of proteinase A synthesis rather than of a mutation in the proteinase A structural gene itself. Since, however, the mutant grew normally on media containing acetate as sole carbon source, its low proteinase A levels cannot simply be due to an inability of 17017 to recover from glucose catabolite repression, a mechanism implicated in the control of proteinase expression in yeast [14,15], but additional regulatory genes must be involved.

'Differentiation-specific' increases in proteinase A activity during sporulation of *Saccharomyces cerevisiae* have been demonstrated and therefore this enzyme was postulated to be essential for the sporulation process [3,4,17]. In order to prove whether the mutation responsible for the proteinase A deficiency in 17017 interferes with ascospore formation, temperature-sensitive segregants derived from crosses between 17017 and wild-type strains were mated with the original mutant 17017 to yield a/α diploids homozygous and heterozygous for the defect. As illustrated in table 2, only diploids heterozygous for

Table 2
Proteinase A specific activities in crude extracts and sporulation ability of diploids homozygous and heterozygous for the proteinase A deficiency

A. Haploid segregants		B. Diploids (haploid segregant × 17017)			
Haploid no.	Proteinase A (U/mg protein)	Diploid no.	Proteinase A (U/mg protein)		Asci (%)
			Vegetative cells	Sporulating cells	
17017$\alpha_2$50	2.66	17017$\alpha_2$50 × 17017	2.31	7.32	56
17017$\alpha_2$51	2.31	17017$\alpha_2$51 × 17017	2.09	7.13	55
17017$\alpha_2$73	0.56	17017$\alpha_2$73 × 17017	0.63	1.20	0
H127.6.2	0.67	H127.6.2 × 17017	0.38	1.26	0
H127.6.3	0.80	H127.6.3 × 17017	0.39	0.48	0

The diploids were crosses of 17017 with temperature-sensitive haploid segregants (mating type α) derived from crosses between 17017 and standard tester strains (see part A of the table). For the assay of proteinase A specific activities, haploids were grown in YM-1 medium for 60 h. Vegetative diploid cells were grown in medium B to ~3.5 A_{600} as detailed in [3]. Sporulating diploids were harvested 20 h after transfer to sporulation medium. The percentage of asci was determined 48 h after transfer to sporulation medium

the mutation exhibited high levels of proteinase A and were able to sporulate, whereas homozygous diploids were not. The small increase in proteinase A specific activity observed after shift of the latter cells to sporulation medium presumably reflected some loss of cell proteins during nitrogen starvation. Apparently, the mutation responsible for the low proteinase A levels in 17017 is incompatible with sporulation.

4. Discussion

At present, the following interpretations for the phenotypic behaviour of mutant 17017 appear possible:

(i) A high activity of proteinase A is required for some critical step(s) in the sporulation process, as for example the maintenance of a high rate of protein degradation or the selective inactivation of various enzymes [3–6]. Preliminary studies suggest that the inactivation of NAD-dependent glutamate dehydrogenase, malate dehydrogenase and isocitrate lyase which occurs after the shift of wild-type cells to sporulation medium [5] is retarded in diploids homozygous for the proteinase A deficiency (unpublished observations);

(ii) Derepression of proteinase A synthesis and sporulation are under some common regulatory control. Former experiments have shown that the transfer to nitrogen-free sporulation medium of yeast growing on acetate as sole carbon source induces a 2–3-fold increase in cellular proteinase A content [3]. By contrast, nitrogen starvation in glucose-containing media provokes only minor changes in the activity of this enzyme (R. J. Hansen, personal communication). One may therefore speculate that both proteinase A synthesis and sporulation are under the coupled control of glucose and nitrogen catabolite repression, the latter being affected in 17017. For the nitrogen and energy balance of the yeast cell, such a dual mode of regulation would be rather economic in ensuring a significant induction of proteinase A in nitrogen-starved and glucose-depleted cells only.

In any case, my experiments with 17017 reveal a striking correlation between proteinase A levels and sporulation ability. Similar observations have been made with asporogeneous strains of *Saccharomyces cerevisiae* [17]. At present, the mechanism and the physiological role(s) of proteinase induction and of protein degradation in sporulating yeast remain obscure. A detailed analysis of mutant 17017 may provide one with means to a clearer understanding of these processes.

Acknowledgements

I am greatly indebted to Dr L. H. Hartwell for several suggestions and the generous supply of the yeast strains and segregants used in this study, to Dr H. Holzer for his interest and support during the course of this work, and to Ms U. Weiser for expert assistance. This work has been supported by the Deutsche Forschungsgemeinschaft, SFB 42.

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