Vol. 95, No. 3, 1980 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
August 14, 1980 Pages 1357-1363

EFFECT OF 5-AMINOLEVULINIC ACID SYNTHESIS DEFICIENCY ON EXPRESSION OF OTHER ENZYMES OF HEME PATHWAY IN YEAST

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Received July 3, 1980

SUMMARY. Some enzymes of the heme pathway have been analysed in the nuclear mutant strains cat11 (hemA-2) and cat7 (hemA-1) of Saccharomyces cerevisiae, both in the rho^+ and rho^- states, and in the mutant ole3 (ole3, rho^+) already described. The mutation leading to the absence of 5-aminolevulinic acid (ALA) synthase activity and to cytochrome deficiency was not expressed in cat11 rho^+, and only partly in cat7 rho^+. The rho^- mutation unmasked the defects associated with the hemA mutations. When expressed, in ole3, cat11 rho^- and cat7 rho^-, the absence of ALA (and heme) synthesis led to a total loss of uroporphyrinogen I synthase activity and to an important increase of coproporphyrinogen III oxidase activity. Genetic analysis showed that hemA-1 and ole3 are two different unlinked loci.

INTRODUCTION. Mutants of Saccharomyces cerevisiae have been described that affect 5-aminolevulinic acid (ALA) synthase, the first enzyme of the heme biosynthetic pathway (1-4). These mutants have partial (2, 3) or complete (1, 4) deficiency of all cytochromes that can be restored to normal by supplementing the growth medium with ALA. This phenotype is under the control of a single recessive nuclear locus that has been referred to as cyd1 (2, 3), ole3 (1) and hem1 (4). In the case of cyd1, the rho^+ strain has a very low level of ALA synthase activity but is only slightly deficient in cytochromes, whereas the rho^- strain is almost completely cytochrome deficient (3). The synergistic effect of the rho^- or a mit^- mutation on the phenotypic expression of the cyd1 defect has been found recently to depend upon glucose repression (5). It has been suggested that the cyd1 cells contain a labile ALA synthase which is partially functional in the rho^+ state but cannot function under respiratory deficiency (3, 5).

We have described two nuclear mutants of S. cerevisiae, cat7 (hemA-1) and cat 11 (hemA-2) isolated in the rho^- state as catalase and cytochrome deficient mutants (6), which lack ALA synthase (7). As previously reported (6, 8), the defect caused by the hemA mutation was not expressed in the rho^+

0006-291X/80/151357-07$01.00/0

cells. The present results extend these preliminary investigations and show that the absence of ALA and/or other heme-precursor in hemA and ole3 strains leads to changes in the expression of two enzymes of the heme pathway. On the other hand evidence will be presented that hemA-1 and ole3 mutations are at different unlinked loci.

MATERIALS AND METHODS. The mutant strain ole3 (a ole3 rho^+) and the parent strain S288C (α mal gal2 rho^+) where kindly provided by Dr R.A. Woods (1). The mutant strains cat7 (α leu1 ade1 hemA-1 rho^-) and cat11 (a ura1 phe hemA-2 rho^-), and their parental strains M/S2-1 (α leu1 ade1 rho^+) and 55R5/3C (a ura1 rho^+) respectively, have been described previously (6,7). The mutant cat7-4B (α leu1 ade1 hemA-1 rho^+) was a segregant from a cross cat7 x IL166-6C (a ura1 rho^+), and the strain cat11-1D (a ura1 leu1 hemA-2rho^+) derived from a cross cat 11 x M/S2-1 (rho^+). The rho^- strains were obtained by ethidium bromide (40 µg/ml) treatment (9). Standard method were used for genetic analysis (10).

Cells were cultured in the following medium : 1 % yeast extract (Difco), 1 % bactopeptone (Difco), 0.5 % Tween 80 + 20 mg/l ergosterol, and 2 % glucose (YPG) or 3 % glycerol (YPgly). 5-aminolevulinic acid (ALA) (Fluka) was eventually added at 30 mg/l. Growth conditions, harvest and preparation of the acellular extract (H) were as described (7).

Spectrophotometric analysis of the cytochromes and tetrapyrrolic pigments content was performed on the whole cells at liquid nitrogen temperature as previously reported (7, 11).

The published procedures (12) were used to measure in the acellular extracts (H) the activities of ALA synthase, porphobilinogen (PBG) synthase and the rate of the overall porphyrins synthesis from ALA or PBG, except for the following modifications : i) the extracts were not dialysed and the assays were run with 4-6 mg protein/ml ; ii) the activity of ALA synthase was measured in the presence of succinate plus glycine and added succinothiokinase purified from yeast and with 8 mM o-phenanthroline instead of EDTA (Felix, F. and Volland, C., unpublished). The coproporphyrinogen III oxidase activity was measured aerobically at 30° with 0.1 to 0.5 mg protein of the acellular extract and 2 µM [^{14}C] coproporphyrinogen III, using the radiochemical test of Grandchamp and Nordmann (13). The activities were expressed as nmole of product/hour/mg protein H.

RESULTS AND DISCUSSION

Cytochrome spectra. The two mutants cat11 and cat7 were originally isolated in the rho^- state and showed deficiency in all cytochromes (6, 7). Two segregants rho^+ have been obtained after crosses with rho^+ wild type strains and their cytochrome spectra are presented in Fig.1. cat11-1D rho^+ when grown on glycerol had almost normal cytochromes as compared with the parental strain 55R5/3C grown on glycerol. When grown on glucose, the addition of ALA to the growth medium increased significantly the cytochrome level. In contrast cytochromes are barely detectable in unsupplemented medium when cat11-1D cells are in the rho^- state. cat7-4B rho^+ and rho^- showed the same behaviour, except that the rho^+ cells had a low cytochrome content when grown on glycerol or glucose in absence of exogenous ALA. The strain ole3 rho^+ was unable to

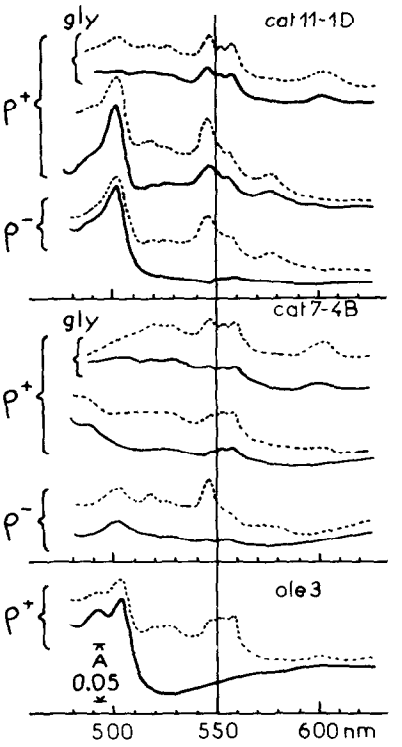

Figure1. Low-temperature absorption spectra of whole cells of the mutant strains cat11-1D, cat7-4B and ole3 in the rho+ and rho⁻ states. The cells were grown in YPG or YPgly (gly) medium, supplemented (- - -) or not (——) with ALA 30 mg/l, and harvested during the last generation of the exponential growth. Spectra were recorded as described (7) with 40 mg dry wt of cells reduced by endogenous substrates.

grow on glycerol and had no detectable cytochromes when grown in unsupplemented medium (Fig.1) as already reported (1).

The three strains, especially cat11-1D and ole3, presented during their exponential phase of growth absorption bands around 490 nm and 500-502 nm, suggesting the presence of oxidized forms of linear or cyclized polypyrrylmethane intermediates, as already mentioned for other heme-mutants of yeast(7).

Enzymes activities of the heme pathway. Some activities of the heme pathway have been measured in acellular extracts (H) obtained from cat11-4B, cat7-4B and ole3 grown in YPG medium, eventually supplemented with ALA, and harvested at the beginning of the last generation of their exponential phase of growth. These activities were compared with the activities measured in the parental strains. The main results (Table 1) are : (i) ALA synthase activity was more than 50 % of wild type level in cat11-1D rho+, but only 10 % at most in cat7-4B rho+. The activity was barely detectable in both strains rho⁻ and ole3. That correlates well with the amount of cytochromes made in vivo,

TABLE I : Enzyme activities of the heme pathway measured in the different ALA synthase-deficient mutants

Strains \ Enzyme activities (nmole/h/mg protein H)		ALA synthase	PBG synthase	overall porphyrins synthesis from ALA	coprogen III oxidase
cat11-1D	rho^+	1.2 - 1.7 (2.1 - 2.4)[a]	0.35 - 0.4 (0.9 - 1.3)	0.05 - 0.06 (0.000)	0.30 —
"	rho^-	0.06 - 0.10 (1.3 - 1.7)	0.3 - 0.6 (1.0 - 1.15)	0.000 - 0.005 (0.000)	5.5 - 6.3 (0.9 - 1.1)
"	" + ALA[c]	—[b]	0.35	0.05 - 0.06	0.16 - 0.18
cat7-4B	rho^+	0.10 - 0.20 (2.1 - 2.3)	0.25 - 0.45 (0.4 - 0.5)	0.02 - 0.025 (0.04 - 0.06)	— (0.18 - 0.20)
"	rho^-	0.00 - 0.05 (1.6 - 1.7)	0.3 - 0.4 (0.4 - 0.6)	0.000 (0.05 - 0.06)	5.0 - 5.5 (0.18 - 0.20)
"	" + ALA	—	0.5 - 0.6	0.05 - 0.055	0.19 - 0.21
ole3	rho^+	0.00 - 0.08 (1.4 - 1.6)	0.2 - 0.3 (0.4 - 0.6)	0.000 (0.04 - 0.05)	6 - 7.5 (0.40 - 0.45)
"	" + ALA	—	0.5 - 0.7	0.04 - 0.05	1.5 - 1.6

a. The values in brackets correspond to the activities measured in the parental strain : 55R5/3C, M/S2-1, S288C for the strains cat11-1D, cat7-4B, and ole3 respectively.

b. Not determined

c. ALA was added in the growth medium : 30 mg/l.

as seen in Fig.1. It appears then, that ALA synthase (and therefore cytochromes) deficiency associated with the hemA mutations was only partly expressed, and to different extents, in cells having retained normal mitochondrial functions and/or genome, and that the rho⁻ mutation unmasked these defects. (ii) PBG synthase activity was about twice lower in mutants as compared to parental strains. It could be restored to wild type values by supplementing the growth medium with ALA in the case of cat7-4B rho⁻ and ole3. (iii) Coproporphyrinogen III oxidase activity was much higher (10 to 20 fold) in mutant cells exhibiting deficiency in ALA and heme synthesis, than in wild type strains or in mutants grown with ALA. Such an higher enzyme activity has been reported in the cytochrome-deficient mutant cyt and in wild type cells grown anaerobically (14), and it has been suggested that this might result from the absence of some end product, as heme, which repressed synthesis of this enzyme. (iv) No synthesis of porphyrins, from ALA of PBG, was observed in the extract obtained from ALA synthesis deficient cells, indicating the absence of at least uroporphyrinogen I (urogen I) synthase activity[1]. The activity was recovered in cells grown with ALA. We found that cycloheximide prevented completely the appearance of urogen I synthase activity caused by the addition of ALA to ole3 growing cells, indicating the participation of cytosolic protein synthesis. These results suggest that the synthesis of this enzyme might be regulated through induction by ALA or by its substrate PBG. Such regulatory mechanism has been described in yeast for some enzymes of the pyrimidine synthesis pathway which are induced in a sequential fashion by intermediate metabolites (15). On the other hand, a sequential induction of early heme pathway enzymes, including urogen I synthase, has been found to occur during erythroid differentiation of mouse Friend virus-transformed cells (16) and of normal cells as well (17 and ref. in 16).

Genetic analysis. Tetrads derived from the crosses cat7 rho⁻ x Il166-6C rho⁺ and cat11 rho⁻ x M/S2-1 rho⁺ have been analysed. All segregants, in the rho⁺ state, grew on glycerol and had cytochromes ; however it was possible to see a 2:2 segregation on the basis of the rates of colonies formation on solid glycerol medium and of the cytochrome content. When the spores were rho⁻, a 2:2 segregation of the cytochrome deficiency was clearly observed. ole3 was crossed with M/S2-1 and the segregants showed a 2:2 segregation of ole3 spectrum to wild type spectrum.

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[1] It must be pointed out that urogen I synthase activity was present in the original mutants cat7 rho⁻ and cat11 rho⁻ (7). The reasons for the loss of this activity in the rho⁻ derivatives of the rho⁺ segregants remain unclear. Also, for unknown reasons, no porphyrins synthesis could be measured in vitro with the strain 55R5/3C as noted before (7).

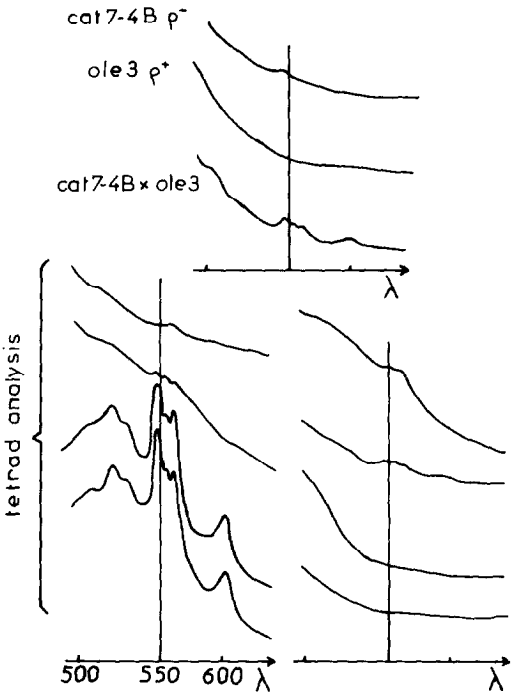

Figure2. Low-temperature whole cells spectra of the different segregants obtained duging the genetic analysis of the mutant strains cat7-4B and ole3. Spectra were run with paste of cells harvested in stationary phase (11). Reduction was achieved by endogenous substrates.

The two mutants ole3 rho$^+$ and cat7-4B rho$^-$ were crossed and the diploid analysed. It showed a very low content of cytochromes, similar to that observed in cat7-4B rho$^+$ (Fig.2), suggesting that ole3 and hemA-1 might be allelic. 14 tetrads from that diploid were analysed for their cytochrome contents and unexpectedly a segregation 6 non-parental ditype : 5 parental ditype : 3 tetratype was found, as illustrated in Fig.2. That means that the two mutations are at different and unlinked loci. Since the diploid was characterized by a mutant phenotype, this suggests some interaction between the two mutant gene products in spite of the fact that each mutation separately was fully recessive. We do not know whether -these two loci are structural genes for two different subunits of ALA synthase, -one locus represents the structural gene for the enzyme and the product of second is necessary for the synthesis or maturation of the enzyme, or its transfer into the mitochondria, -the products of the two genes play a regulatory role. The answer to these questions will await a better characterization of ALA synthase both in wild type and in mutant strains.

In CONCLUSION, our results i) show that the development of a fully active ALA synthase is under the control of two distinct unlinked nuclear genes ; ii) suggest the possibility for urogen I synthase to be regulated by induction by ALA or PBG and for coproporphyrinogen III oxidase to be controled by repression by heme. These problems are currently under investigation.

ACKNOWLEDGEMENTS. We wish to thank Drs B. Grandchamp and Y. Nordmann for their help with the coproporphyrinogen oxidase assay and for the gift of [^{14}C] coproporphyrin III. This work was supported partly by the Centre National de la Recherche Scientifique (E.R. 186), the Delegation Generale à la Recherche Scientifique et Technique (77.7.0257) and the University Paris VII, partly by the Polish Academy of Science (project 0.9.3.1.).

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