Modified uroporphyrinogen decarboxylase activity in a yeast mutant which mimics porphyria cutanea tarda

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The isolation of a new mutant Sm1 strain of yeast, *Saccharomyces cerevisiae*, is described: this strain was partially defective in haem formation and accumulated large amounts of Zn-porphyrins. Genetic analysis showed that the porphyrin accumulation was under the control of a single nuclear recessive mutation. Biochemical analysis showed that the main porphyrins accumulated in the cells were uroporphyrin and heptacarboxyporphyrin, mostly of the isomer-III type. The excreted porphyrins comprised mainly dehydroisocoproprophyrin. Analysis of uroporphyrinogen decarboxylase activity in the cell-free extract revealed a 70–80% decrease of activity in the mutant and showed that the relative rates of the different decarboxylation steps were modified with the mutant enzyme. A 2–3-fold increase in 5-aminolaevulinic synthase activity was measured in the mutant. The biochemical characteristics of the Sm1 mutant are very similar to those described for porphyria cutanea tarda.

Although the enzymic events leading to the synthesis of haem are well established in higher organisms, the control mechanisms regulating this pathway are less completely understood (Granick & Beale, 1978). Most of the information about the regulation of haem biosynthesis was provided by the studies of porphyrias, which are disorders of porphyrin metabolism either inherited in humans or chemically induced in experimental animals and tissue cultures (Sassa & Kappas, 1981; Granick & Beale, 1978). They are characterized by specific partial enzyme deficiencies and the overproduction and accumulation of specific haem precursors, namely the substrates of the defective enzymes.

We have undertaken a systematic study of haem synthesis in the yeast *Saccharomyces cerevisiae*, a well-documented unicellular eukaryotic organism. The same sequence of catalytic steps was found as described in higher organisms (Labbe-Bois & Labbe, 1978). Yeast offers many opportunities for genetic studies, which may provide valuable insights into the regulatory mechanisms of haem

synthesis. Of particular interest is the class of mutants deregulated or partially defective in individual steps of haem formation. Such mutants are expected to synthesize haemoproteins and respire, and to accumulate porphyrin precursors and fluoresce under u.v. light. Yeast mutants accumulating substantial amounts of octa- to tetracarboxyporphyrins have previously been described (Pretlow & Sherman, 1967; Arrese *et al.*, 1982), which do not exhibit major cytochrome deficiencies, except for one which lacked cytochrome oxidase (Arrese *et al.*, 1982). However, no enzymic analysis was performed to determine the exact nature of the defect causing porphyrin accumulation in the cells. In this paper, we describe the isolation of new yeast mutants partially affected in uroporphyrinogen decarboxylase (uroporphyrinogen carboxy-lyase, EC 4.1.1.37) activity, the phenotype bearing striking similarities to that reported for inherited or experimental porphyria cutanea tarda.

Experimental

Strains

The mutant strains were isolated from the wild-

Abbreviation used: h.p.l.c., high-performance liquid chromatography.

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type strain SP4 (α *leu1 arg4 rho*⁺) (Bilinski *et al.*, 1980). G121 mutant strain (α *ura2 hem12-1*) has been described previously (Urban-Grimal & Labbe-Bois, 1981).

Genetic analysis

Standard media and procedures were used for crossing, sporulation and tetrad analysis (Sherman *et al.*, 1974).

Growth conditions and preparation of cell-free extracts

Cells were grown in 3 litres of 1% yeast extract (Difco), 1% Bacto-peptone (Difco) and 2% glucose (YPG medium) or 2% ethanol (YPE medium). Since the strain G121 requires Tween 80 and ergosterol for growth (Urban-Grimal & Labbe-Bois, 1981), the media were supplemented with 0.2% (w/v) Tween 80 and 30 mg of ergosterol/l to give the same growth conditions for all strains. Cells were grown at 30°C in 6-litre spherical flasks with vigorous magnetic stirring and aeration (2 litres/min per litre of solution). To obtain the cell-free extracts, cells were harvested at the beginning of the last generation of the exponential phase of growth, washed with water and broken with glass beads (Labbe-Bois & Volland, 1977).

Determination of cytochrome, haem and haem precursors

Qualitative and preliminary spectrophotometric analyses of the cytochrome and tetrapyrrolic pigments were performed on whole cells at liquid-N₂ temperature (Labbe-Bois & Volland, 1977). Cytochrome and haem concentrations were determined spectrophotometrically as described (Urban-Grimal & Labbe-Bois, 1981). Intracellular 5-aminolaevulinate and porphobilinogen were extracted and estimated colorimetrically with modified Ehrlich reagent (Urban-Grimal & Labbe-Bois, 1981).

Intracellular porphyrins were exhaustively extracted with 3M-HCl and measured spectrophotometrically; since these extracts contained a mixture of different porphyrins, the Soret-band absorption coefficient used was calculated from those of the individual porphyrins present, taking into account their proportions as determined by the h.p.l.c. technique described below. The HCl extracts were evaporated under vacuum; the residues were esterified with methanol/H₂SO₄ (19:1, v/v) and left overnight in the dark at room temperature. The porphyrin esters were extracted into chloroform and evaporated to dryness and redissolved in known volumes of chloroform before analysis by h.p.l.c. A Waters Associates μ -Porasil column (300 mm $\times$ 3.9 mm; 10 μ m particle size) was used with a Waters Associates model

6000A solvent-delivery system, a model U6K injector and a Unicam model SP.6-500 spectrophotometer operated at 404 nm coupled to a Itelec model IF 5000 recorder. The peak areas were measured with an integrator (Delsi model ICAP 5), and quantification was performed after calibration with standard mixtures of porphyrin esters (Porphyrin Products, Logan, UT, U.S.A.). The elution solvent was ethyl acetate/cyclohexane (11:9 or 1:1, v/v) at a flow rate of 1.8 ml/min. Fractions containing individual porphyrin esters were collected, and the solvent was evaporated. Each porphyrin ester was further analysed for purity by h.p.l.c. and by its spectral properties. The uroporphyrin, hepta-, hexa- and penta-carboxyporphyrin and coproporphyrin esters were hydrolysed overnight in 0.1 ml of 6M-HCl, evaporated to dryness, and then the isomer composition was determined with reverse-phase h.p.l.c. by using an acetonitrile/phosphate buffer system and a Waters C18- μ Bondapak column (300 mm $\times$ 3.9 mm) at a flow rate of 1.2 ml/min as described by Kushner *et al.* (1982). Retention times of the I and III isomer peaks were compared with those of authentic standards of uroporphyrins I and III, coproporphyrins I and III, hepta-, hexa- and penta-carboxyporphyrins I and III obtained either from Porphyrin Products or purified from the urine of a patient with porphyria cutanea tarda (kindly provided by Dr. H. de Verneuil, Laboratoire de Biochimie, Hôpital Louis Mourier, 92701 Colombes, France).

Excreted porphyrins were measured spectrophotometrically in the cell-free culture medium made 3M with HCl. For their identification, the porphyrins were extracted with talc from the cell-free culture medium adjusted to pH 4.5 with acetic acid, esterified, transferred to chloroform (Urban-Grimal & Labbe-Bois, 1981) and then separated and quantified by h.p.l.c. as described above.

Enzyme assays

The activities of seven enzymes of haem biosynthesis were measured in cell-free extracts of the parental and mutant strains. The assays were run at 30°C, and activities are expressed as nmol of product/h per mg of protein: protein was measured by the Lowry method, with bovine serum albumin as standard. Published procedures were used to measure the activities of 5-aminolaevulinate synthase and porphobilinogen synthase (Urban-Grimal & Labbe-Bois, 1981). Coproporphyrinogen oxidase was measured (Grandchamp & Nordmann, 1977) with [¹⁴C]coproporphyrin prepared as described by Chambon & Labbe (1982). Protoporphyrinogen oxidase (Camadro *et al.*, 1982) and ferrochelatase (Camadro & Labbe, 1981) were also assayed. The rate of the overall por-

phyrin synthesis from porphobilinogen to protoporphyrin (Urban-Grimal & Labbe-Bois, 1981) was taken as a measure of uroporphyrinogen I synthase activity, since it is the rate-limiting step of that enzymic sequence.

The assay of uroporphyrinogen decarboxylase was based on methods previously described [Straka *et al.* (1982), Elder & Wyvill (1982) and references cited therein] by the following procedure. Solutions of commercial uroporphyrins I and III (about 5 mM in 5 mM-KOH) were reduced with 3% sodium amalgam under N_2 , and stock solutions of uroporphyrinogen (0.2–1 mM in 0.2 M-potassium phosphate buffer, pH 6.8, containing 50 mM-di-thiothreitol) were kept frozen (-20°C) for several weeks in small glass tubes under a drop of pure paraffin oil without reoxidation. Unreduced porphyrin in the stock solution (usually 2–4%) was determined by recording the $A_{405.5}$ ($\epsilon_{\text{mM}} = 541$) as quickly as possible after dilution with 0.5 M-HCl. Porphyrinogen concentration was then calculated from the total porphyrin present after reoxidation with 0.2 M- I_2 , followed by dilution with 0.5 M-HCl. The uroporphyrinogen decarboxylase reaction mixture (final volume 5 ml) contained cell-free extract (20–40 mg of proteins), passed through a small Sephadex G-25 column (PD 10; Pharmacia) to eliminate endogenous porphyrins (San Martin de Viale *et al.*, 1977), in 0.1 M-potassium phosphate buffer containing 0.1 mM-EDTA and 10 mM-di-thiothreitol (pH 6.8). After preincubation at 30°C for 10 min in a small test tube, 5 units of catalase (Boehringer), 20 units of glucose oxidase (Serva) and glucose (final concn. 10 mM) were added to achieve anaerobiosis. The reaction was started 1 min later by the addition of uroporphyrinogen (5–15 μM final concn.), and the reaction mixture was then immediately withdrawn into a blackened 5 ml Combitip (Eppendorf) syringe with an Eppendorf Multipipette; the Combitip syringe was then stoppered with a pipette tip previously sealed in a flame. The Combitip syringe was placed in a 30°C water bath, and 1 ml samples were delivered by the Multipipette at desired time intervals into 0.25 ml of ethyl acetate/acetic acid (3:1, v/v) to stop the reaction. The porphyrinogens were oxidized by 25 μl of 0.2 M- I_2 and the mixtures were evaporated almost to dryness under a stream of air. Subsequently the porphyrins, converted into their methyl esters, were quantified and analysed by h.p.l.c. as described above. The recovery of porphyrins for each point (usually about 90%) was calculated by comparing the sum of uroporphyrin and reaction products recovered with the known amount of substrate added. Appropriate controls and reagent blanks were included in the assay. The uroporphyrinogen decarboxylase activity was estimated as the sum of nmol of hepta-, hexa-, penta-

and tetra-carboxyporphyrins formed in 1 h. No difference in the pattern of accumulated decarboxylation products was found within the range 1–7 mg of protein/ml of assay and with a substrate concentration range of 7–14 μM .

Enzyme activities estimated in vivo

The rates of synthesis of haem and haem precursors *in vivo*, based on the known cell generation time, were calculated from measurements of the intracellular pools of porphyrin intermediates, cellular haem concentration and the amounts of porphyrins excreted during growth. For example, the overall accumulation and excretion of all intermediates as well as of haem were taken into account when calculating 5-amino-laevulinate synthase activity. These rates are expressed as nmol of product formed/h per mg of protein (300 ± 30 mg of protein was recovered in the cell-free extract from 1 g dry wt. of cells).

Errors

All determinations of enzyme activities and concentrations of intracellular and excreted metabolites were made with an accuracy of $\pm 5\%$ and were performed in the same culture: the whole experiment was duplicated. The absolute values varied by only 10–15% from one experiment to another.

Results

Isolation and genetic analysis of the mutants

The original SP4 strain was grown in YPE medium to the stationary phase of growth and then plated on solid YPE medium (10000 cells/plate). Plates were irradiated for 60 s with u.v. radiation (254 nm; 0.188 mJ/mm²). This treatment resulted in a 1% survival rate. The mutagenized cells were incubated for 5 days at 30°C and the surviving colonies were examined under u.v. at 366 nm for red fluorescence. Of the 50000 colonies tested, only 166 colonies fluoresced. After four passages on YPE solid medium, only three of these 166 colonies continued to fluoresce. These fluorescing mutants were designated Sm1, Sm2 and Sm3. The whole-cell spectra of each of the mutants showed a vast accumulation of Zn-porphyrins, characterized by two absorption peaks at about 540 and 575 nm: the whole-cell spectra of the Sm1 mutant strain grown on either glucose or ethanol media are typical, and are compared with those of the wild-type strain (Fig. 1). The genetic analysis of all three mutants revealed that the porphyrin accumulation was under the control of a single nuclear recessive mutation. Furthermore, the mutants Sm1, Sm2 and Sm3 were allelic; therefore, we chose one of them, Sm1, for a detailed biochemical analysis.

Table 1. *Accumulation and excretion of haem precursors by the parent and mutant strains*

Measurements were performed as described in the Experimental section with cells harvested at different points during the two last generations of their exponential growth on either glucose or ethanol medium. Since the values obtained did not vary by more than the accuracy of the methods ($\pm 5\%$), results shown represent single assays in one experiment. Numbers in parentheses are the percentages of isomer I.

Strain	Growth conditions	Generation time (h)	Intracellular 5-amino-laevulinic acid ($\mu\text{mol/g dry wt.}$)	Intracellular porphobilinogen ($\mu\text{mol/g dry wt.}$)	Haem (nmol/g dry wt.)	Porphyrins (nmol/g dry wt.)	Percentage of each porphyrin*									
							8	7	6	5	4a	4	3	2	proto	2
Wild-type SP4	Glucose (2%)	2.1	0.88	<0.05	70	Intracell.	20.3 (50)	7.8 (1)	10.2	5.5 (100)	0	52.4 (3)	2.3	1.5		
						Excreted	0	0	1.2	6.2	0	76	6.6	10		
							4.75					(3)				
						Intracell.	26 (32)	7.6 (1)	17.6 (15)	12.3 (100)	0	35 (6)	0.5	1		
Mutant Sm1	Glucose (2%)	3.15	1.64	<0.05	≤ 3	Excreted	0	0	1.5	1.8	0	60.7 (27)	27	9		
							13									
						Intracell.	80.2 (18)	14.1 (1)	4 (80)	1	0.4 (30)	0.2 (10)	0	0		
						Excreted	13.7 (16)	4.8	6.8	10.9	40	18 (70)	4	1.7		
	Ethanol (2%)	4.5	5.05	<0.05	65	Intracell.	64.8 (9)	25 (1)	6.1 (80)	1.8 (30)	0.5	1.8 (6)	0.02	0		
						Excreted	2	1.7	7.4	6.3	56.6 (74)	15.2	9.3	1.5		
							35									

* 8, 7, 6, 5, 4a, 4, 3 and 2 refer respectively to octa- (uro), hepta-, hexa-, penta-, tetra- (dehydroisocopro), tetra- (copro), tri- (hardero) and di- (proto) carboxylic porphyrins.

The Sml strain was crossed with mutants totally blocked in different steps of haem biosynthesis (Urban-Grimal & Labbe-Bois, 1981), and the diploids were analysed by running low-temperature spectra of intact cells. It appeared that the Sml mutant did not complement the mutant *hem12-1* (G121), which is totally deficient in uroporphyrinogen decarboxylase. The Sml × G121 diploid had the Sml phenotype. Therefore the mutated allele in Sml strain was designated *hem12-2*.

Biochemical analysis of the Sml mutant

As shown in Table 1, the growth rate of the mutant cells was diminished compared with the parent strain, but the yield reached the same value. The longer generation time might be due to the accumulation of porphyrins, which are known to be toxic to the cells. More 5-aminolaevulinate was found in the mutant cells, but there was no accumulation of porphobilinogen. Haem synthesis was very low in the mutant cells when grown fermentatively on glucose, and they also accumulated less porphyrins than did cells grown respiratively on ethanol: these results are consistent with the whole-cell spectra (Fig. 1). Although much

Fig. 1. Low-temperature absorption spectra of whole cells of the parent SP4 (a) and mutant Sml (b) strains. The cells were grown on glucose (Glu) or ethanol (Eth) medium and harvested during the last generation of the exponential growth. Spectra were recorded as described (Labbe-Bois & Volland, 1977) on 40 mg dry wt. of cells. Reduction was performed by endogenous substrates.

Table 2. Specific activities of some enzymes of the protohaem-synthesis pathway measured in cell-free extracts of the parent and mutant strains. Cell-free extracts were obtained from cells harvested at the beginning of the last generation of the exponential growth on glucose or ethanol. Enzyme activities were measured *in vitro* and estimated *in vivo* as described in the Experimental section. Results given are the averages for four determinations made in two separate experiments. The ranges for the specific activities were 15% of the average values. Values in parentheses are activities estimated *in vivo*.

Strain	Growth conditions	Activity (nmol of product/h per mg of protein)							
		5-Amino-laeuvinate synthase	Porpho-bilinogen synthase	Uroporphy-rinogen I synthase	Uroporphy-rinogen I decarboxylase	Uroporphy-rinogen III decarboxylase	Coproporphy-rinogen oxidase	Protoporphy-rinogen oxidase	Ferro-chelatase
Wild-type SP4	Glucose (2%)	3.6 (2.7)	0.84 (0.65)	0.08 (0.123)	0.198 (0.002)	0.255 (0.120)	0.15 (0.11)	2 (0.11)	4.5 (0.11)
	Ethanol (2%)	3.2 (3.3)	1.0 (1.0)	0.105 (0.230)	0.277 (0.004)	0.365 (0.225)	0.16 (0.21)	2.85 (0.21)	6 (0.21)
Mutant Sml	Glucose (2%)	6.45 (2.6)	0.58 (0.43)	0.025 (0.08)	0.031 (0.003)	0.045 (0.026)	8.6-10 (0.01)	3.8 (0.00)	5 (0.00)
	Ethanol (2%)	8.5-10 (5.4)	0.85 (0.82)	0.135 (0.187)	0.049 (0.005)	0.106 (0.108)	0.2 (0.07)	3.15 (0.05)	9 (0.05)

Fig. 2. Pattern of porphyrin accumulation and excretion in the mutant *Sm1* during growth on glucose (Glu) or ethanol (Eth). Values are taken from Table 1. ▨, Type-I isomers; □, type-III isomers; ▤, isomer not determined; 8, 7, 6, 5, 4a, 4, 3, 2 indicate uro-, hepta-, hexa-, penta-carboxy-, dehydroisocopro-, copro-, tricarboxy- (hardero) and protoporphyrins; H, indicates total haem. See the text for full experimental details.

porphyrin was accumulated by the *Sm1* strain, the sum of haem plus porphyrins was the same in both the parent and the mutant cells grown on either glucose or ethanol, which indicates a similar production of tetrapyrroles by both the parent and *Sm1* strains. The nature of the porphyrins made by *Sm1* strain is presented in Table 1 and Fig. 2. The main porphyrins accumulated in the cells were uroporphyrin and heptacarboxyporphyrin. The excreted porphyrins comprised mainly dehydroisocoproporphyrin, which was identified by its absorption spectrum (Elder, 1972), its chromatographic behaviour (Evans *et al.*, 1976) and its mass spectrum ($M = 708$) (F. de Maack is acknowledged for the desorption chemical ionization mass spectra obtained by using a Riber R 10–10 mass spectrometer). Dehydroisocoproporphyrin arises from the oxidative decarboxylation, catalysed by

coproporphyrinogen oxidase, of the 2-propionate side chain of pentacarboxyporphyrinogen III (Elder & Evans, 1978). The porphyrins formed were mostly of the isomer III type, indicating that uroporphyrinogen III synthase activity was not significantly impaired in the mutant.

Such a pattern of porphyrin accumulation and excretion, observed throughout growth, strongly suggests a defect in uroporphyrinogen decarboxylase activity. This was confirmed by measurements of the activity of this enzyme in the cell-free extracts. Graphic representation of the rates of accumulation of the different reaction products, catalysed by both the normal and mutant enzymes (Fig. 3), show the complexity of the reaction, as has been previously described for both liver and erythrocyte uroporphyrinogen decarboxylases (Garcia *et al.*, 1973; San Martin de Viale *et al.*,

Fig. 3. Accumulation of decarboxylation products from uroporphyrinogen I (a) and uroporphyrinogen III (b) when incubated anaerobically with cell-free extracts obtained from wild-type parent and *Sml* mutant grown on ethanol medium

The conditions were described in the Experimental section, with $14\mu\text{M}$ -uroporphyrinogen I, $15\mu\text{M}$ -uroporphyrinogen III, and 5.2mg of protein/ml of assay for wild-type extract or 7.6mg protein/ml of assay for mutant extract. Δ , Heptacarboxyporphyrinogen; $\circ$, hexacarboxyporphyrinogen; $\diamond$, pentacarboxyporphyrinogen; ∇ , coproporphyrinogen. The presence of heptacarboxyporphyrin at zero time is due to a small contamination of the substrate uroporphyrin by this porphyrin. The pattern of porphyrin accumulation was highly reproducible, so the results corresponding to one experiment are reported.

1977; Smith & Francis, 1979, 1981). However, the data presented clearly show that the relative rates of the different decarboxylation steps were modi-

fied in the mutant. The first decarboxylation of both uroporphyrinogens I and III appeared to be diminished, together with the second decarboxyla-

tion of uroporphyrinogen III and the third decarboxylation of uroporphyrinogen I. These results correlate well with the pattern of porphyrins accumulated in the mutant cells (Fig. 2). That the same kinetics were observed with the normal enzyme over a wide range of enzyme concentration (results not shown) argues for a qualitative alteration of the catalytic activity of the mutant enzyme rather than a marked decrease in the amount of normal enzyme: a decrease in decarboxylase activity to only about 20–30% of that of the wild type could be calculated from the total of reaction products made in 1 h in the cell-free extracts (Table 2). In both the parent and mutant strains, uroporphyrinogen III was decarboxylated faster than uroporphyrinogen I and the activity was higher in cells grown on ethanol than on glucose. Since the rates of product formation were not linear over the 60 min period studied, it might be inferred that these measurements are not truly valid values; however, a diminution of activity approximating to 80% in glucose-grown cells and 50% in ethanol-grown cells was also estimated *in vivo* (Table 2, values in parentheses). Therefore there is strong evidence that enzyme assays *in vitro* reflected the situation *in vivo*.

The deficiency in uroporphyrinogen decarboxylase activity, and therefore in haem synthesis, led to changes in the activities of other enzymes of the pathway as shown in Table 2. Uroporphyrinogen I synthase activity was lower in the glucose-grown mutant cells and porphyrin production was linear for a shorter period of time in the mutant than in the parent strain. Such a phenomenon has been described for other *hem* mutants (Bilinski *et al.*, 1981; Urban-Grimal & Labbe-Bois, 1981). The fact that a higher activity of uroporphyrinogen I synthase was estimated *in vivo* in all strains might reflect some disorganization of enzyme function after breakage of the cells. Also, the large increase in coproporphyrinogen III oxidase activity in Sml cells grown on glucose was not surprising, since it has previously been observed in haem-less mutants (Labbe-Bois *et al.*, 1980; Urban-Grimal & Labbe-Bois, 1981). The activities of protoporphyrinogen synthase, protoporphyrinogen oxidase and ferrochelatase did not differ significantly in the mutant and parent strains.

The 2–3-fold increase in 5-aminolaevulinate synthase activity is noteworthy, since it is the first time that a significant increase of 5-aminolaevulinate synthase activity has been shown to occur in a yeast mutant affected in haem biosynthesis. It might be taken as evidence for repression of 5-aminolaevulinate synthase by the end-product haem, as shown for liver cells (Granick & Beale, 1978); however, such an increase was not observed in the mutants completely deficient in haem

(Urban-Grimal & Labbe-Bois, 1981). In the Sml strain, on the other hand, there was no relationship between the 5-aminolaevulinate synthase activity and the amount of haem synthesized by the cells. The regulatory event underlying the increase in 5-aminolaevulinate synthase activity is unclear at present.

Discussion

The results reported in this paper show that a modified uroporphyrinogen decarboxylase activity appeared to be the primary defect in the Sml mutant of *S. cerevisiae*. The nature of the porphyrins accumulated in or excreted by intact cells was fully consistent with the behaviour of the enzyme studied in cell-free extracts. That Zn-porphyrins rather than porphyrinogens accumulated in the cells probably resulted from non-enzymic oxidation of porphyrinogens and non-enzymic insertion of Zn into the porphyrins.

The biochemical characteristics of the Sml mutant are very similar to those described for porphyria cutanea tarda. This disorder of porphyrin metabolism in higher organisms is characterized by a massive hepatic accumulation and increased urinary excretion of uroporphyrin and heptacarboxyporphyrin III, together with excretion in faeces of isocoproporphyrin and its congeners, owing to a partial deficiency in uroporphyrinogen decarboxylase activity (Elder, 1977). This porphyria, inherited in humans, can be induced in experimental animals by polyhalogenated aromatic hydrocarbons (Elder, 1978). More recently, it was reported that these chemicals act by inhibiting the catalytic activity of uroporphyrinogen decarboxylase (Sinclair *et al.*, 1983), at the level of the two first decarboxylations (Kawanishi *et al.*, 1983), but without changing the amount of immunoreactive enzyme protein (Elder & Sheppard, 1982). It is noteworthy that the mutation in the Sml strain also led to an alteration in the two first decarboxylation steps of uroporphyrinogen III. However, nothing can be currently said about the molecular lesion leading to this defect. The genetic data described previously (Urban-Grimal & Labbe-Bois, 1981) and in the present paper strongly suggest that a single enzyme specified by the locus HEM12 catalyses the four decarboxylations of both uroporphyrinogen I and III in *Saccharomyces cerevisiae*, as has also been shown with the enzyme purified from human erythrocytes (De Verneuil *et al.*, 1983). Although uroporphyrinogen I decarboxylation involves a random sequence of decarboxylations (Jackson *et al.*, 1977), there are indications that uroporphyrinogen III is decarboxylated by a preferred sequential series of reactions starting with the acetic acid moiety on

the D ring and followed, in order, by those on the A, B and C rings (Jackson *et al.*, 1980). However, the mechanism of the reactions is not yet known and, in particular, whether the decarboxylations take place at two or possibly more catalytic sites (Garcia *et al.*, 1973; San Martin de Viale *et al.*, 1977; Smith & Francis, 1979, 1981; De Verneuil *et al.*, 1980). A detailed genetic and biochemical study of other yeast mutants recently isolated in our laboratories, including the Sm2 and Sm3 mutants, which have a similar phenotype to that of Sm1, may help us to understand the mode of action of uroporphyrinogen decarboxylase.

That mutants from yeast could be isolated which mimic both inherited and experimental porphyria cutanea tarda strongly suggests that the functioning of the haem pathway in yeast and in higher organisms is very similar. It is hoped that the use of yeast mutants as models will make possible a study of the defects underlying the different types of human porphyrias and the environmental factors that affect the expression of these defects.

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