Correlation between the rate of proteolysis of mitochondrial translation products and fluidity of the mitochondrial inner membrane in *Saccharomyces cerevisiae* yeast

Alteration of the rate of proteolysis under glucose repression

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Our previous results [Kalnov, Novikova, Zubatov & Luzikov (1979) *FEBS Lett.* **101**, 355–358; *Biochem. J.* **182**, 195–202] suggested that in yeast the mitochondrial translation products localized in the mitochondrial inner membrane are rapidly broken down by a proteolytic system inherent in the membrane. In the present work, it is demonstrated that, on glucose repression in undividing cells of *Saccharomyces cerevisiae*, there is no proteolysis of the mitochondrial translation products. This effect is not likely to be associated with lower activity of the proteolytic system of the mitochondrial inner membrane. Nor is the cessation of proteolysis due to qualitative changes in the composition of mitochondrial translation products. What repression does cause is a considerable alteration in the physical state (i.e. structure of the lipid bilayer) of the mitochondrial inner membrane; this was established by experiments involving lipid-soluble spin probes. The conclusion is reached that the rate of proteolysis of mitochondrial translation products in the mitochondrial inner membrane depends on the physical state of the membrane, which in its turn is controlled by the relative content of unsaturated fatty acid chains in the mitochondrial phospholipids.

It is known (for review see Schatz & Mason, 1974; Tzagoloff *et al.*, 1979) that the mitochondrial inner membrane is assembled from polypeptides synthesized on both cytoplasmic and mitochondrial ribosomes. Such mitochondrial translation products include subunits I, II, and III of cytochrome *c* oxidase, apocytochrome *b*, and one or several subunits of the membrane sector of H⁺-ATPase (Schatz & Mason, 1974; Tzagoloff *et al.*, 1979; Kolarov *et al.*, 1981).

Several years ago (Bakalkin *et al.*, 1978; Kalnov *et al.*, 1979a) it was established in this laboratory that the mitochondrial translation products synthesized in yeast cells in the presence of cycloheximide, i.e. under interrupted cytoplasmic translation, undergo proteolysis with a half-life of 20–60 min depending on the growth phase. As was

shown by later experiments, this degradation is due to the action of proteinase(s) associated with the mitochondrial inner membrane (Kalnov *et al.*, 1979b; Novikova *et al.*, 1981).

The significant variation in the half-life of mitochondrial translation products for the different growth phases of yeast (Kalnov *et al.*, 1979b) was interpreted to indicate the existence of a certain mechanism controlling the rate of their proteolysis. Our present study of the proteolysis in undividing cells of *Saccharomyces cerevisiae* under glucose repression has revealed the existence of a direct correlation between the rate of this process and fluidity of the mitochondrial inner membrane, which in its turn is controlled by the content of unsaturated fatty acid chains in mitochondrial phospholipids.

Materials and methods

The strain of *S. cerevisiae* used and the growth conditions have been described previously (Luzikov *et al.*, 1971).

Cell respiration was measured with a PO-4 polarograph (Radiometer, Denmark) at 30°C with

Abbreviations used: Ac-TyrOEt, acetyltyrosine ethyl ester; SMP, submitochondrial particles; SDS, sodium dodecyl sulphate; I_{12,3}, 2-(3-carboxypropyl)-4,4-dimethyl-2-tridecyl-3-oxazolidinyloxyl; I_{1,14}, 2-(14-carboxytetradecyl)-2-ethyl-4,4-dimethyl-3-oxazolidinyloxyl; CAT₁₂, 4-dodecyltrimethylammonium-1-oxyl-2,2,6,6-tetramethylpiperidine bromide.

the use of a Clark electrode. The assay medium contained 1 mM-EDTA, 0.25% glucose and 0.05 M-phosphate buffer (pH 7.4). To measure respiration, 2 mg of the yeast cells was added to 1 ml of the assay medium. The respiration of the cells was expressed in nmol of O/min per mg wet wt. of cells.

Incorporation of radioactive label into the mitochondrial translation products *in vivo* was performed in the presence of cycloheximide as described previously (Bakalkin *et al.*, 1978), except that 100 ml of the cell suspension was supplemented with 100 μ Ci of L-[4,5- 3 H]leucine (sp. radioactivity 250 mCi/mmol; Amersham) and, in the double-labelling experiments, 40 μ Ci of L-[U- 14 C]leucine (sp. radioactivity 330 mCi/mmol; Amersham). Incorporation of the label for gel electrophoresis was performed in the same way, but the amounts of [3 H]leucine and cycloheximide were increased to 3 mCi/l and 100 μ g/ml, respectively.

Breakdown of the mitochondrial translation products *in vivo* and *in vitro* was followed as described previously (Bakalkin *et al.*, 1978; Kalnov *et al.*, 1979b).

The experiments on glucose repression of the yeast were carried out in the following way. The yeast was grown to the early stationary growth phase, after which the concentration of cells in the suspension was raised to 5% (w/v). The cells suspended in the growth medium were incubated under aerobic conditions at 35°C in the presence of 10% (w/v) glucose for 0.5, 1.0, 1.5 and 2.0 h. In the control experiments, the thickened cell suspension was incubated in the absence of glucose under the same conditions for 2.0 and 3.0 h. After a 2 h glucose repression, the cells were washed with growth medium containing no glucose (derepression) and incubated in the standard growth medium (Luzikov *et al.*, 1971) for 0.5, 1.0, 1.5 and 2.0 h. The label was always added 0.5 h before the end of the incubation periods indicated above.

The cell homogenate was prepared as described by Tzagoloff (1969), and the ultrasonic SMP according to Schatz (1968). The SMP did not display any measurable antimycin A-insensitive NADH-cytochrome *c* reductase and kynurenine hydroxylase activities or activities of proteinases A and B. Hence, the SMP fraction was practically free from endoplasmic reticulum, mitochondrial outer membranes or vacuoles.

For measuring the rate of hydrolysis of Ac-TyrOEt, the method of Jusić *et al.* (1976) was employed; it was modified for our purposes as described previously (Novikova *et al.*, 1981).

Cytochrome *c* labelled with NaB 3 H $_4$ was obtained according to Means & Feeney (1964). The [3 H]-cytochrome *c* hydrolase activity was assayed as described in Novikova *et al.* (1981).

The protein assay was performed according to

Lowry *et al.* (1951) with the use of bovine serum albumin as a standard.

Lipids were extracted from SMP by the method of Folch *et al.* (1957). The plates for micro-t.l.c. on 5–10 μ m silica gel (Woelm) were prepared as described by Svetashev & Vaskovsky (1972).

For the resolution of phospholipids, a system of solvents suggested by Rouser (1973) was used. The phospholipids were revealed with the help of a developing agent (see Vaskovsky *et al.*, 1975).

Methyl esters of the fatty acids were obtained as described by Metcalfe *et al.* (1966). The contents of various fatty acids were determined in a Varian model 3700 gas chromatograph; column, 0.4 cm $\times$ 100 cm; phase SP-2330 on Chromosorb W (100–200 mesh); column temperature 150–220°C, carrier He at 2 ml/min, injection and detector temperature 250°C. The lipid-soluble, spin-labelled probes I $_{12,3}$, I $_{1,14}$ and CAT $_{12}$:

were used for testing thermoinduced structural changes in the SMP membrane.

I $_{12,3}$ and I $_{1,14}$ were manufactured by Syva (Palo Alto, CA, U.S.A.). CAT $_{12}$ was synthesized by Dr. R. Melhorn (University of California, Berkeley, CA, U.S.A.). The probes were dissolved in ethanol; their final concentration in the SMP suspension was 10 $^{-4}$ M. The final concentration of ethanol in the preparation after introduction of the probe did not exceed 1%. A special study that had been carried out previously showed that such a concentration of ethanol does not entail any side-effects, e.g., it does not alter the structure of lipids, or change the ATPase activity of the membrane preparation of (Na $^+$, K $^+$)-ATPase (Tverdislov *et al.*, 1980), or the kinetics of electron transport in chloroplasts (Ruuge *et al.*, 1977; Tikhonov & Ruuge, 1979). The e.p.r. spectra were taken in a Varian E-4 e.p.r. spectrometer with the following instrument settings: field modulation frequency, 100 kHz; microwave power, 10 mW; microwave frequency, 9.17 GHz; modulation amplitude, 1 G; time constant, 0.1–0.3 s; scan rate, 12.5 G/min. Temperature of samples was regulated with a variable temperature controller, whose error did not exceed 0.5°C. In the experi-

ments with the $I_{12,3}$ spin probe, the modulation amplitude was increased to 2 G to enhance the accuracy of the measurements.

For the electrophoretic separation of the mitochondrial translation products in the presence of 0.1% SDS, the system described by Weber & Osborn (1969) was employed, the only difference being that 12.5% polyacrylamide gel was used. Electrophoresis was carried out at room temperature under constant current (3 mA/gel) for 18 h. SMP were treated before application onto the gel as described by Ibrahim *et al.* (1973); 200 μ g of protein was applied per tube. To elute the label, 1 mm slices of gel were added to 0.5 ml of 0.3% SDS and incubated at 35°C for 15 h; then 10 ml of the following scintillant was added: 2,5-diphenyloxazole (4 g/l), 1,4-bis(5-phenyloxazol-2-yl)benzene (0.2 g/l); naphthalene (60 g/l) in dioxan/ethanol (4:1, v/v).

Radioactivity was measured in a Nuclear-Chicago mark III liquid-scintillation spectrometer. The counting efficiency was 24–27% for ^3H and 63–67% for ^{14}C . The total amount of the label eluted from the gel was 90% of that applied. For calibration of the gel, the following reference proteins were used: ovalbumin (M_r 45 000), chymotrypsinogen A (M_r 25 000), myoglobin (M_r 17 800) and cytochrome *c* (M_r 12 400).

Results

Experimental conditions

The experiment was carried out with a thickened 5% (wet wt./v) suspension of yeast grown to the early stationary phase; this prevented division of the cells after the addition of glucose to the incubation medium. As established previously (Luzikov *et al.*, 1973), thickening *per se* does not cause any noticeable biochemical or morphological changes in the mitochondria.

Fig. 1 shows that on addition of glucose (final concn. 10%) to the incubation medium the respiration rate of the cells gradually goes down to reach 50% of the initial level 3 h later. During the subsequent 3 h the respiration remains more or less the same.

As the content of cellular protein in these conditions increased by only 10–15%, the alteration in the respiratory activity could have hardly been caused by the 'dilution' of the respiratory chain components by other proteins; rather, it must have been entailed by disorganization of the respiratory chain, evidenced, among other things, by a 25–30% decrease of cytochrome *aa₃* content. The content of cytochrome *b* in the cells did not change in these conditions.

When transferred to a medium devoid of glucose, the cells displayed an increasing respiration rate that reached the initial level in 3 h.

Fig. 1. Respiration of yeast cells during glucose repression and derepression

A_0 , respiration of the cells before glucose addition; A , respiration of the cells during the incubation of yeast in the absence of glucose (x), during glucose repression (●) and derepression (○). The initial respiration of the cells (A_0) was 44 nmol of $\text{O} \cdot \text{min}^{-1} \cdot (\text{mg wet wt.})^{-1}$. The respiration of yeast remained sensitive to 1 mM-KCN throughout the incubation.

Incorporation of [^3H]leucine into mitochondrial translation products under glucose repression

Mitochondrial translation products were labelled by a 0.5 h pulse in the presence of cycloheximide immediately after the yeast was thickened or after 0.5, 1.0, or 1.5 h of incubation in the glucose-containing medium. As follows from Fig. 2, under these conditions the incorporation of [^3H]leucine into mitochondrial translation products was enhanced in the course of glucose repression. It should be noted that the thickening of the cells *per se* also stimulated the incorporation, but to a much lesser degree (Fig. 2).

In non-repressed yeast, a certain increase in the label in the mitochondrial translation products was observed if the isolation of SMP (but not labelling) had been performed in the presence of phenylmethanesulphonyl fluoride, a serine proteinase inhibitor, which, as shown in our previous work (Kalnov *et al.*, 1979a,b), blocks the proteolysis of these polypeptides *in vivo* and *in vitro*. If three inhibitors of this process, i.e. leupeptin, phenylmethanesulphonyl fluoride and *p*-chloromercuriphenyl sulphonate, were used, the content of the label in the mitochondrial translation products reached the same level as after a 2 h glucose repression followed by isolation of SMP in the absence of the proteinase inhibitors (Table 1). This fact suggested that proteolysis of mitochondrial translation products was inhibited by glucose repression.

Fig. 2. Incorporation of $[^3\text{H}]$ leucine into mitochondrial translation products

Incorporation of $[^3\text{H}]$ leucine ($100\mu\text{Ci}/100\text{ml}$) was carried out in the presence of cycloheximide ($25\mu\text{g}/\text{ml}$) for 30 min during the incubation of the cells in the presence (●) and in the absence (○) of glucose. At the time intervals indicated, aliquots of yeast suspension were collected and the radioactivity of isolated mitochondria was measured (Kalnov *et al.*, 1979b).

Table 1. Effect of glucose repression and proteinase inhibitors on the level of $[^3\text{H}]$ leucine in mitochondrial translation products

The proteinase inhibitors were added 5 min before the end of the pulse and also to all the solutions employed for the isolation of SMP.

Conditions of label incorporation	Radioactivity (d.p.m./mg of protein)
Control without additions	6900
Control + $100\mu\text{M}$ -phenylmethane-sulphonyl fluoride	7935
Control + $100\mu\text{M}$ -phenylmethane-sulphonyl fluoride + leupeptin ($2\mu\text{g}/\text{ml}$) + 1mM - <i>p</i> -chloro-mercuriphenyl sulphonate	10000
Glucose repression	12040

As was shown by electrophoresis in SDS/polyacrylamide gels of the solubilized SMP preparations; both glucose repression (Fig. 3) and the proteinase inhibitors induce an increase in the relative content of $[^3\text{H}]$ leucine in polypeptides of apparent M_r 30000–50000. Ibrahim *et al.* (1973) reported lower incorporation of label into mitochondrial translation products on glucose repression. These experiments, however, were carried out under an entirely different set of conditions and the results, therefore, defy comparison.

Fig. 3. Effect of glucose repression on the distribution of radioactivity in SMP proteins after incorporation in vivo of $[^3\text{H}]$ leucine in the presence of cycloheximide

(a) SMP were isolated from yeast cells labelled with $[^3\text{H}]$ leucine in the presence of cycloheximide ($100\text{mg}/\text{l}$) and chloramphenicol ($4\text{g}/\text{l}$). (b, c) The same in the presence of cycloheximide only. Incorporation was carried out for 30 min in the absence of glucose (a, b) or after a 2 h incubation of the cells in the presence of 10% glucose (c). In all cases $200\mu\text{g}$ of SMP protein was applied onto the gel. Treatment of the samples and electrophoretic resolution were carried out as described in the Materials and methods section.

Degradation of mitochondrial translation products under glucose repression of yeast

The degradation of mitochondrial translation products has been studied in pulse-chase experi-

ments with thickened yeast after 0.5, 1.0, and 2.0 h of repression. The results shown in Fig. 4 demonstrate the time-course of $^3\text{H}_{\text{m, synth.}}$ after a 30 min pulse of [^3H]leucine in the presence of cycloheximide. The value of $^3\text{H}_{\text{m, synth.}}$ reflects the content of [^3H]leucine in the mitochondrial translation products at certain moments of the chase with post-incorporation and recycling of the label being taken into account (for details see Bakalkin *et al.*, 1978).

It is obvious from Fig. 4(a) that after 2.0 h of glucose repression the proteolysis of the mitochondrial translation products ceases. It is noteworthy that in non-repressed thickened yeast, proteolysis (i.e., the release of the label into the trichloroacetic acid-soluble fraction) is completed within 2 h (Fig. 4a, curve 4). The data on the breakdown of the mitochondrial translation products in the non-repressed and repressed yeast explain the difference in the recorded levels of [^3H]leucine incorporated into these proteins before and 2.0 h after the addition of glucose to the incubation medium (see Fig. 2).

As shown in Fig. 1, cell respiration can be fully restored during 3 h of derepression, which is well in accord with the data of Jayaraman *et al.* (1966). In parallel, the rate of proteolysis of mitochondrial translation products increases; but it does not achieve the initial level after 3 h of derepression (Fig. 4b).

The data on the breakdown of the mitochondrially made polypeptides *in vivo* (Fig. 4) show a good correlation with the results for the degradation

of these polypeptides in mitochondria isolated from the yeast after incorporation of [^3H]leucine in the presence of cycloheximide. These experiments (see Fig. 5) have shown that in the mitochondria of repressed cells, the release of [^3H]leucine into the

Fig. 5. Effect of glucose repression on the breakdown of mitochondrial translation products *in vitro*

The breakdown of mitochondrially made polypeptides in isolated mitochondria was measured as described previously (Kalnov *et al.*, 1979b). The radioactivity of mitochondrial proteins at time 0 of incubation was assumed to be 100%. ●, 0 h; ○, 1 h; △, 1.5 h; □, 2 h of repression.

Fig. 4. Effect of glucose repression on the breakdown of mitochondrial translation products *in vivo*

The breakdown of mitochondrially made polypeptides was assayed as described previously (Bakalkin *et al.*, 1978).

$^3\text{H}_{\text{m, synth.}}$ was expressed as a percentage of the values corresponding to the time 0 of the chase. (a) Repression of yeast: ●, 0 h; ○, 0.5 h; △, 1 h; □, 2 h. (b) Derepression of yeast: ○, 0 h; ●, 0.5 h; △, 1 h; □, 2 h.

trichloroacetic acid-soluble fraction proceeds at a much lower rate than in the mitochondria of the non-repressed cells.

Cytochrome c hydrolase activity of SMP isolated from the non-repressed and repressed yeast

One of the plausible reasons for the retarded proteolysis of mitochondrial translation products in repressed yeast is the decrease of the proteolytic activity or the disappearance of proteinase(s) from the inner membrane of mitochondria. To verify this possibility, a procedure for the measurement of the proteolytic activity in SMP involving the use of cytochrome *c* labelled with NaB^3H_4 was elaborated.

As shown in Fig. 6, the hydrolysis of $[\text{}^3\text{H}]$ -cytochrome *c*, which manifests itself as accumulation of the label in the trichloroacetic acid-soluble fraction, has a more or less similar rate in the presence of SMP from the non-repressed and repressed (for 2 h) yeast. Similar results were obtained with the Ac-TyrOEt hydrolase activity of SMP (Fig. 6).

We have previously reported data on the sensitivity of cytochrome *c* hydrolase and Ac-TyrOEt hydrolase activities, as well as of the proteolysis of mitochondrially made polypeptides, to certain proteinase inhibitors (Novikova *et al.*, 1981). Both with

the non-repressed and repressed yeast, all the three processes are inhibited by the same substances, i.e. phenylmethanesulphonyl fluoride, *p*-chloromercuriphenyl sulphinate, leupeptin, antipain and chymostatin (Novikova *et al.*, 1981). This leads to the idea that they are performed by one and the same proteinase(s) of the mitochondrial inner membrane. If so, the rate of hydrolysis of $[\text{}^3\text{H}]$ -cytochrome *c* can be taken to measure the activity of the mitochondrial proteolytic system that breaks down the mitochondrial translation products.

It should be noted that the sensitivity to inhibition of the cytochrome *c* hydrolase and Ac-TyrOEt hydrolase activities hardly ever changed under glucose repression.

The results reported in this section allow the conclusion to be made that the retardation of the proteolysis of mitochondrial translation products under glucose repression is not likely to be associated with the lower activities of proteinases of the mitochondrial inner membrane.

Altered physical state of the mitochondrial inner membrane under glucose repression

Fig. 7 shows the e.p.r. spectra of the $\text{I}_{12,3}$ spin probe added to SMP suspensions isolated from

Fig. 6. Effect of glucose repression on $[\text{}^3\text{H}]$ -cytochrome *c* hydrolase and Ac-TyrOEt hydrolase activities of yeast SMP

Cytochrome *c* hydrolase and Ac-TyrOEt hydrolase activities are expressed as a percentage of those in SMP of non-repressed yeast. These values were, respectively, $2.5 \times 10^{-2} \text{ nmol} \cdot \text{h}^{-1} \cdot (250 \mu\text{g of protein})^{-1}$ and $30 \text{ nmol} \cdot \text{min}^{-1} \cdot (\text{mg of protein})^{-1}$. O, Cytochrome *c* hydrolase activity; ●, Ac-TyrOEt hydrolase activity.

Fig. 7. E.p.r. spectra of spin probe $\text{I}_{12,3}$ in SMP suspension

SMP were suspended in 30 mM-Tris/HCl buffer (pH 7.6) in the presence of 0.2 mM-EDTA. Concentration of SMP was 3 mg of protein/ml; concentration of the probe was 10^{-4} M . The e.p.r. spectra were taken as indicated in the Materials and methods section. (a) SMP of yeast grown up to the early stationary phase; (b) SMP of yeast after 2 h glucose repression.

repressed and non-repressed yeast. These e.p.r. signals are recorded as the superposition of two signals, i.e. that of the major portion of the probe, whose paramagnetic group is localized in the hydrophobic region of the membrane (extrema 1–5) and the other, less significant, portion whose paramagnetic group has a polar (aqueous) environment (extrema 6 and 7). The e.p.r. spectra suggest fast anisotropic rotation of the paramagnetic group in the hydrophobic region of the membrane. If one takes into account the molecular dimensions of the probe, one may conclude that the paramagnetic group is buried at a distance of approx. 0.8 nm from the surface of the membrane, where the hydrocarbon chains of the phospholipids have a relatively ordered structure (for details see McConnell, 1976; Ruuge *et al.*, 1977; Tikhonov & Ruuge, 1979).

It is known (Tikhonov & Ruuge, 1979; Gaffney, 1976; Margolis *et al.*, 1980; Griffith & Jost, 1976) that the temperature dependences of the parameters of the spin probes in the biomembranes, i.e. the correlation time of the rotational diffusion, τ_c , and the order parameter, S , which is largely determined by the mobility of the spin probe and its orientation to the local anisotropy axis of the membrane (McConnell, 1976), have characteristic inflexions which correspond to the phase transition temperature; the latter depends, among other things, on the fatty acid composition of the membrane lipids

(McConnell, 1976; Margolis *et al.*, 1980; Griffith & Jost, 1976; Schreier *et al.*, 1978). These data are most demonstrative if presented in terms of the temperature dependence of the e.p.r. spectral parameter $2T'_{||}$ (Ruuge *et al.*, 1977; Tikhonov & Ruuge, 1979). The $2T'_{||}$ parameter (the distance between 'external' extrema 1 and 5 of the e.p.r. spectrum) of the probe localized in the membrane characterizes the order and mobility of the hydrocarbon chains of the phospholipids in SMP. We can state, with a great degree of accuracy, that $2T'_{||}$ is proportional to the order parameter, S (Gaffney, 1976). The temperature dependence of $2T'_{||}$ of the $I_{12,3}$ probe for the non-repressed yeast is presented in Fig. 8(a). It can be seen that this dependence has characteristic inflexions at 5, 15 and 53°C that become obvious if the curve is approximated by several straight lines. The inflexions are readily reproducible. For instance, it can be seen in Fig. 8(a) that the curves for the different control SMP preparations (the open and the filled circles) are almost the same.

In the case of SMP from repressed yeast (Fig. 8b), the inflexion points in the temperature dependences of $2T'_{||}$ are shifted by 8°C towards higher temperatures (13 and 23°C). These differences may be ascribed to an alteration in the lipid composition of the mitochondrial inner membrane, e.g., a decrease in the relative content of unsaturated hydrocarbon

Fig. 8. Temperature dependence of the $2T'_{||}$ parameter of the e.p.r. spectrum of spin probe $I_{12,3}$ in SMP suspension (a) SMP of yeast grown up to the early stationary phase; (b) SMP of yeast after 2h glucose repression. For details see Fig. 7. Open and filled circles stand for different SMP preparations.

chains in the phospholipids under glucose repression (see the following section).

Comparison of Figs. 8(a) and 8(b) shows that under glucose repression the slope of the temperature dependence of the $2T'_{\parallel}$ parameter also becomes different. The latter may be due to the fact that under glucose repression the rearrangements in the lipid bilayer of the mitochondrial inner membrane are not associated solely with the altered mobility of the hydrocarbon chains in the lipids; the other important factors are the structure of the polar 'heads' of the phospholipids (Margolis *et al.*, 1980) and the protein composition of the membrane (Griffith & Jost, 1976). The change of the charge on the surface of SMP should theoretically bring about redistribution of the charged probe molecules between the membrane and the aqueous phase. Our study has shown, however, that glucose repression hardly causes any changes in the distribution of the probes between the polar and hydrophobic regions of the mitochondrial inner membrane. The results that support this conclusion were obtained with the $I_{1,14}$ spin-labelled derivative of stearic acid, which at intracellular pH carries a negative charge, and also with the CAT_{12} probe, which has a positive charge. In the case of the $I_{1,14}$ probe, the information about its distribution between the membrane and the aqueous phase can be obtained from the comparison of the two components of the spectrum, i.e., H , which is due to the probe the paramagnetic group of which is localized in the membrane, and P , which is due to

the probe localized in the aqueous phase (McConnel, 1976). It is obvious from Fig. 9(a) that SMP from the repressed and non-repressed cells have a similar H/P ratio.

The same conclusion can be drawn from the analysis of the relationship between the components of the e.p.r. spectrum of the CAT_{12} spin probe. It is seen in Fig. 9(b) that glucose repression hardly ever changes the ratio of the values of any components 1–5, which are due to the probe whose paramagnetic group is localized in the membrane, or in components 6 or 7, which are due to the CAT_{12} whose paramagnetic group is in the aqueous phase. These data indicate that the surface charge of the membrane does not change under glucose repression.

Phospholipid composition and ratio of unsaturated to saturated fatty acids in the total phospholipid fraction of SMP under glucose repression

As was shown by t.l.c. on silica gel, the total phospholipid fractions of the non-repressed and repressed yeast contain an identical set of phospholipids (results not shown). At the same time, the degree of saturation of the fatty acids, determined as the ratio of the total amount of unsaturated to the total amount of saturated fatty acids (K), noticeably changes after addition of glucose to the incubation medium. For the phospholipid fraction of the non-repressed yeast $K = 6.5$ and for the repressed yeast $K = 4.0$. It follows from Table 2 that the difference should largely be ascribed to the

Fig. 9. E.p.r. spectra of spin probes $I_{1,14}$ (a) and CAT_{12} (b) in SMP suspension

(A) SMP of yeast grown up to the early stationary phase; (B) SMP of yeast after 2 h glucose repression. For details see Fig. 7.

Table 2. *Effect of glucose repression on the composition of fatty acids in the total phospholipid fraction of SMP*

SMP prepared from:	Relative content of fatty acids (%)			
	C _{16:0}	C _{16:1}	C _{18:0}	C _{18:1}
Yeast grown to the early stationary phase	8.9	50.4	4.4	36.3
Yeast after 2 h of glucose repression at the early stationary phase	11.8	48.1	8.1	32.0

higher relative content of palmitic and stearic acids. The contents of palmitoleic and oleic acids do not significantly change in the course of the glucose repression.

Discussion

The present work has been undertaken to elucidate how proteolysis of mitochondrial translation products is controlled in the mitochondrial inner membrane. It has been shown, first and foremost, that the breakdown of these polypeptides is almost completely suppressed under glucose repression.

One can think of three reasons for the altered rate of proteolysis in the membrane: (i) altered activity of proteinase(s); (ii) altered set of hydrolysable proteins; (iii) altered physical state of the membrane. Assessment of the hydrolytic activity towards certain substrates (Ac-TyrOEt and cytochrome *c*) allowed the assumption to be made that the retarded proteolysis of mitochondrially made polypeptides is hardly due to the lower activity of proteinases in the mitochondrial inner membrane.

The second suggestion also does not seem plausible in the case under consideration. Indeed, a practically complete cessation of the proteolysis of mitochondrial translation products (see Fig. 4a) would imply that the normal set of hydrolysable polypeptides is entirely altered during glucose repression, so that the major components of this set disappear and the label is concentrated in the minor or newly evolving components. However, SDS/polyacrylamide-gel electrophoresis of solubilized SMP from repressed yeast revealed only some changes in the relation between mitochondrially synthesized polypeptides as compared with the control. Both in control and under glucose repression, after the electrophoretic resolution of the mitochondrial translation products the highest radioactivity was detected in components with apparent M_r values of 56 000, 49 000, 43 000, 33 000, 22 000, 14 500, and 8000. These data are roughly the same as those of Ibrahim *et al.* (1973) and Mian *et al.* (1973), who found that glucose repression changes only the relative content of the individual mito-

chondrially made polypeptides, with the set of these polypeptides remaining the same.

As to the third suggestion, the results of our e.p.r. study suggest that glucose repression alters the order and mobility of phospholipid hydrocarbon chains of the mitochondrial inner membrane. It is known that in homogeneous lipid systems, liposomes or multilayers of synthetic phospholipids unidirectionally oriented on a support (Schreier *et al.*, 1978; Margolis *et al.*, 1980), the temperature dependence of the $2T'_{1/2}$ parameter has a single distinct inflexion that corresponds to the phase transition temperature. This temperature, in its turn, depends on the structure of the polar 'head' of the lipid and on the length and the degree of saturation of the hydrocarbon chains (Margolis *et al.*, 1980). The lower the degree of saturation of the hydrocarbon chains the lower is the phase transition temperature, which manifests itself as a shift to lower temperature of the inflexion point in the temperature-dependence curve of the $2T'_{1/2}$ parameter. In heterogeneous systems the temperature dependence of this parameter usually has two inflexions (see Margolis *et al.*, 1980).

Comparison of Figs. 8(a) and 8(b) shows that on glucose repression the inflexions on the temperature dependence of the $2T'_{1/2}$ parameter of the $I_{12,3}$ spin probe shift towards higher temperatures. These changes are well in agreement with the fact that the ratio of the total amount of the unsaturated to the total amount of saturated fatty acids in the total lipid fraction of SMP from the non-repressed yeast is greater ($K = 6.5$) than in the SMP from the repressed yeast ($K = 4.0$). All this suggests (see McConnell, 1976; Schreier *et al.*, 1978) higher ordering and lower mobility of the phospholipid hydrocarbon chains of SMP membranes from the repressed yeast.

The altered physical state of SMP membranes could well be the reason for the retardation or cessation of the proteolysis of mitochondrial translation products under glucose repression. In fact, since both proteinase and mitochondrially made polypeptides are localized in the membrane (Kalnov *et al.*, 1979b), they would interact the more the better are the conditions for lateral and rotational diffusion. The latter, in its turn, largely depends on the order and mobility of the phospholipid hydrocarbon chains in the membrane (McConnell, 1976). Of course, final proof of this suggestion would require direct measurements of the rates of diffusion in the mitochondrial inner membrane of the mitochondrially made polypeptides on the one hand, and proteinases on the other, for control and repressed yeast. Such measurements cannot be carried out at the present time.

We cannot also exclude other explanations for the observed phenomena. It is admissible, for instance,

that the mutual position of the mitochondrially made polypeptides and the proteinases is changed owing to general alterations in the membrane architecture under glucose repression; or that the proportions between particular mitochondrial proteinases are altered while their overall activity towards exogenous substrates remains constant, and the like.

With all this, the correlation between the rate of the proteolysis of mitochondrial translation products and the physical state of the mitochondrial inner membrane appears so natural that it could be supposed to have a universal character and to hold true for a wide range of physiological conditions. Data in support of this suggestion are being sought.

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