ACCELERATION OF CYTOCHROME OXIDASE SYNTHESIS SPECIFIC TO ZYGOTES FROM CROSSES BETWEEN COMPLEMENTARY RESPIRATORY DEFICIENT MUTANTS OF S. cerevisiae.

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A cross between two haploid respiratory deficient mutants of yeast, able to complement (i.e. cross between a neutral cytoplasmic mutant and a chromosomal one, or between two non allelic chromosomal mutants), yields zygotes and a diploid progeny able to synthesize a complete respiratory system. Such cells are called "grande". (Chen, Ephrussi and Hottinguer, 1950 ; Slonimski, 1953 ; Sherman and Slonimski, 1964 ; Jakob, 1965).

In some conditions, the development of the respiratory activity of such zygotes and of their immediate progeny is different from that of the remote progeny of the same genotype (several cell generations later, which we call "established grande"). The rate of the development is much higher in the former case (newly formed "grande") than in the latter (established "grande"), Jakob (1965).

Two hypotheses are available to explain such a result, either this high respiratory activity is due to a particulary rapid de novo synthesis of the respiratory enzymes, or to a rapid high molecular activity of presynthesized enzymes without any increase in their rate of synthesis. The results presented in this paper allow us to choose between these alternatives for the case of cytochrome oxidase.

Results -

Crosses were made to obtain synchronous zygotes (Jakob, 1965). In this study, the zygotes arise from a cross between a haploid auxotrophic

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chromosomal mutant, p_5 p^+ (SG2-1C) and a cytoplasmic neutral one, $\underline{P}$ p^- (C 982-19dA_1) (Jakob, 1965). The zygotes are prototrophic. The mating mixture contains zygotes and a majority of auxotrophic haploid, non mated parental cells. The heterogeneous cell suspension is grown aerobically at 25 C. in minimal medium containing three per cent glucose and samples are taken off after various times of growth (generation time $\sim$ 2 hours). The washed cell suspension is then :

1) plated on different media (Jakob, 1965) to determine the number of prototrophic cells (zygotes and their progeny) and of auxotrophic parental cells.

2) the respiration of the cells is measured in the Warburg apparatus. The measurement is done at 28 C. in a buffer (sodium phosphate-potassium phtalate - succinic acid 0.1 M at pH 4.3 = P.P.S.) in the absence of growth with ethanol as the sole energy source. The O_2 uptake measured during the first hour under such conditions reflects predominantly the basal respiratory metabolism of the cells immediately after their harvesting from the glucose growth medium.

3) the cytochrome oxidase activity is determined. The ice-cold water washed yeast is ground with glass beads in a Nossal apparatus (Nossal 1953) 1 x 30 seconds (Somlo, 1962) in buffer Tris (hydroxy-methyl) amino-methane (Tris) 0.05 M at pH 7.2. From the supernate after centrifugation (1 200 g x 10 minutes) the particulate fraction, sedimenting in 20 minutes at 23 000 g (M fraction), is isolated. The M fraction is washed once with the same buffer centrifuged again during 20 minutes at 23 000 g, and resuspended in the buffer. The cytochrome oxidase activity which is associated with this fraction M is immediately measured by spectrophotometric measures of the oxidation rate of ferrocytochrome c at 28 C (Smith, 1954 ; Smith and Conrad, 1956 ; Sels, 1962). The cytochrome c of horse heart Sigma type III, reduced at 98% approximately by Duolite S10 resin, Chemical process Company, Redwood City, California, was used.

In the studied cross, after 2 hours of growth, the respiration of the prototrophic zygotes and their progeny was 200 μl O_2/h/10^8 "grandes" colony forming centers. After 6 hours of growth ($\sim$3 cell generations) this value was approximately the same but the activity of the cytochrome oxidase has doubled (cf. table I). The cytochrome-oxidase activity of an "established grande", grown under the same conditions and showing the same respiration, never attains such high values.

In a second type of experiment the cell suspensions are studied during aeration in the absence of growth. Samples are taken at various times from the glucose growth medium washed, and suspended in buffer

(P.P.S. 0.1M, pH = 4.3) with ethanol as the sole carbon and energy source.

Under these conditions the respiratory metabolism is derepressed and the Q O_2 values increase considerably. A convenient measure of this derepression is given by the acceleration of the rate of O_2 uptake (i.e. AO_2 Slonimski, 1955);we compared here this acceleration with the evolution of the cytochrome oxidase activity associated with fraction M.

The results of such an experiment are summarized in fig. 1. The increase in the rate of respiration of the zygotes and their immediate progeny is exceptional large. This high AO_2 corresponds to a large increase of the cytochrome-oxidase activity of the extracts. This activity is 360 millimicromoles of ferrocytochrome c oxidised/min/10^8 c.f.c. "grande" after 6 hours of aeration (cf. fig. 1). This is far higher than that found, under the same conditions when a "grande" from the remote progeny of the zygote (established "grande" H.J. 17A of the same genotype) is studied. The activity in this latter case is never higher than 40 millimicromoles of ferrocytochrome c oxidised/min/10^8 c.f.c. "grande".

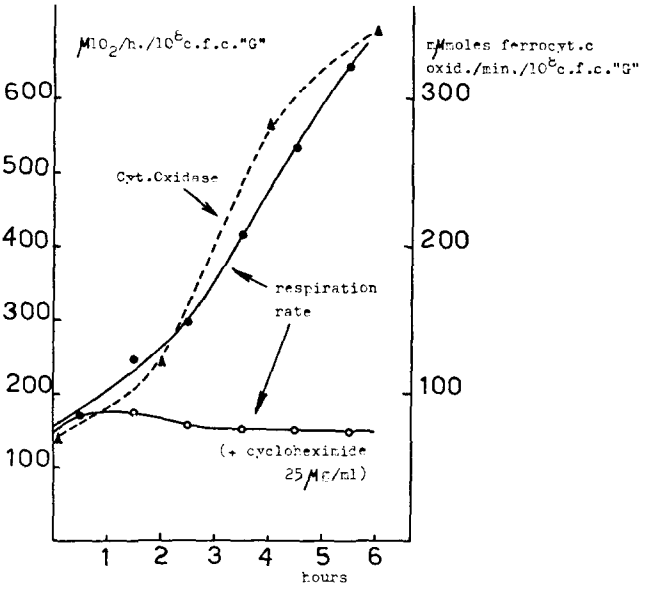

Fig. 1. - The respiration rate with ethanol as sole carbon and energy source (μl O_2/h/10^8 c.f.c. "grandes" is measured during 6 hours in the Warburg apparatus without growth (PPS buffer). The zygotes of the cross p5 p^+ x P p^- neutral were previously grown in aerobic culture in glucose-minimal medium for 3 hours. The cytochrome oxidase activity (m moles of ferrocytochrome c oxidized/min/10^8 c.f.c. "grandes") associated with the M fraction (see text) is determined after various times of aeration in PPS buffer-ethanol.

TABLE I - Development of respiration of the zygotes (cross p_5 p^+ x P p^- neutral) and of an established "grande" during aerobic growth in glucose - minimal medium.

	Time of aerobical growth in minimal medium + 3% glucose (hours)	Number of "grandes" colony forming centers (c.f.c.)/ml in the culture	Rate of respiration on ethanol μl O_2/h/10^8 c.f.c. "grandes" measured during the first hour in the Warburg apparatus.*	Cytochrome-oxidase mμ moles ferrocytochrome c /min/10^8 c.f.c. "grandes"**	Cytochrome Oxidase/ μl O_2
Zygotes and their immediate diploid progeny : "newborn grandes"	0	1.2×10^6	8.1	0	
	2	2.3×10^6	171	31.8	0.186
	4	3.6×10^6	163.5	50.4	0.308
	6	6.7×10^6	198	60.2	0.304
Remote progeny of zygotes : "established grandes"	6	6.0×10^6	195	24.7	0.127

* The parental, non fused respiratory deficient cells present in the studied samples, use up a very small quantity of O_2.

** The cytochrome oxidase activity is evaluated by the product k_1 (cyt.c)o, of the velocity constant of the reaction of order 1 and of the initial concentration of ferrocytochrome c (30 μM- 100% of ferrocytochrome). The extracts of the respiration deficient parental cells catalyse, with a very low rate, the oxidation of ferrocytochrome c, the results are corrected for the very weak activities attributable to the parental cells.

The direct examination with the Cary 15 spectophotometer (Claisse, Péré, Slonimski, 1967), of yeast pellets at the temperature of liquid nitrogen would allow us to demonstrate a net synthesis of the pigment characterized by its adsorption band at 602-3 mμ after reduction with sodium hydrosulfite. A precise analysis of the kinetics of formation of cytochromes (a + a3) at various times was not possible because of the very low proportion of synchronous zygotes present in the analyzed cell populations (7% in the experiment described in Table II). However the spectroscopic data are sufficient to establish clearly the de novo synthesis of cytochrome oxidase. The concentration of the cytochrome oxidase measured on the yeast pellet, 1 mm thick, is deduced from the difference of absorbance between the maximum peak at 602-3 mμ and a baseline obtained by joining the minima of absorption at 575 mμ and 630 mμ ($\Theta^{(a+a3)}_{1\ mm}$). The threshold for the detection corresponds to approximately 15% of established "grandes" in the mixture, when artificial mixtures of "grandes" and mutant cells are tested.

TABLE II -

	Hours of aeration in buffer PPS-ethanol	(a + a3)* 1 cm
Zygotes and their immediate diploid progeny	0	<0.8
	6	2.9 ± 0.6
	6 (+ cycloheximide 25 μg/ml	<0.8
	15	3.4 ± 0.6
Established "Grande" (H.J. 17A)	0	0.53 ± 0.05
	6	0.94 ± 0.04
	6 (+ cycloheximide 25 μg/ml)	0.48 ± 0.05
	15	0.81 ± 0.02

* $\Theta^{(a+a3)}_{1\ cm}$ is calculated for a yeast pellet 1 cm thick.

Table II shows the results obtained on pellets of (1) the zygotes and their immediate progeny grown for 4 hours (~2 generations) in minimal glucose medium and then aerated in buffer containing ethanol and of (2) strain H.J. 17A (established "grande" of same genotype) under the same conditions.

The data show clearly that during the first hours of aeration in PPS-ethanol buffer, cytochromes (a + a3) are synthesized in the zygotes and in their immediate progeny with a rate several times higher than that observed in their remote progeny. $\Theta^{(a + a3)}_{1\ cm}$ increases from 0.8 to 3.0 in the first case and from 0.53 to 0.94 in the second one. The AO_2 is also three times higher in the zygotes than in their remote progeny. It should be noted that the same "established grande" grown in complete glucose medium and harvested in the stationary phase of aerobic growth shows a $\Theta^{(a + a3)}_{1\ cm}$ of 1.40 ± 0.05 and that after 6 hours of aeration in PPS buffer-ethanol this $\Theta^{(a + a3)}_{1\ cm}$ does not exceed 1.85.

We can conclude that a de novo synthesis of cytochrome (a + a3) and a rapid increase of QO_2 are associated. This conclusion is supported by the fact that inhibition of protein synthesis by cycloheximide (25 µg/ml Fukuhara, 1966) strongly inhibits the increase in QO_2 and the synthesis of cytochrome oxidase (cf. table II). Established "grandes" on the other hand, do not show this accelerated de novo synthesis during respiratory adaptation when the previous growth was in anaerobiosis.

After the cross of two complementing respiration deficient mutants, i.e. when the genetic determinants (chromosomal and cytoplasmic) required for the synthesis of the respiratory enzymes are brought together in the same cytoplasm (zygotes), the regulation mechanism of the cytochrome-oxidase and probably that of the other respiratory enzymes, is quite exceptional. The newly formed "grandes" are able to synthesize the cytochrome oxidase at a rate several times higher than that observed normally. This "transitory acceleration" of respiration is observed in all studied zygotes and in their immediate progeny arising from two complementing respiratory deficient mutants. This exceptional behavior raises the problem : what is the nature of the regulation mechanism which controls the synthesis of respiratory enzymes in a "grande" yeast.

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