Changes in the Activities of Respiratory Enzymes during the Aerobic Growth of Yeast on Different Carbon Sources

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1. Unlike yeast cells grown on glucose (0.9%), cells grown on galactose aerobically or anaerobically as well as cells grown on very low glucose concentrations (0.09%) have been found to possess mitochondria. 2. Synthesis of respiratory enzymes was less in the presence of glucose than galactose. When either sugar was consumed, synthesis of these enzymes increased. 3. A possible mechanism is suggested for the repression of mitochondrial formation by glucose by means of a 'high-energy' substance easily derived from glucose.

The effect of growth conditions on the respiratory ability of yeast and especially the effect of the carbon source metabolized on the enzyme content of the yeast cell and the development of mitochondria has been examined by Polakis, Bartley & Meek (1964). Glucose has been shown to be a repressor of the respiratory and citric acid-cycle enzymes under anaerobic and aerobic conditions, as well as of the development of true mitochondrial structures by the yeast cell.

Prompted by the differences of the oxidative ability of cells, observed by Strittmatter (1957) in cells grown aerobically on glucose and galactose and by Tustanoff & Bartley (1964b) in cells grown anaerobically, we have undertaken a study of the structural make-up of cells grown aerobically and anaerobically on galactose, and have made a comparison of the activities of respiratory enzymes in cells grown on different carbon sources. The results of this study, which show that galactose-grown cells, unlike the cells grown on glucose, contain mitochondria at all stages of growth, are presented in this paper. In the light of these results and those of Polakis & Bartley (1965), a possible mechanism of repression of the respiratory enzymes and of mitochondrial formation by a 'high-energy' substance easily obtained by the metabolism of glucose is proposed.

MATERIALS AND METHODS

The sources of chemicals, the maintenance and growth of yeast, the disruption of cells, the gel filtration of the cell-free extract and the analytical methods used were as described in the preceding paper (Polakis & Bartley, 1965).

The anaerobic medium for growth and the preparation of

cells for electron microscopy were as described by Polakis *et al.* (1964).

The measurement of the activities of the enzymes of the respiratory chain were carried out with the same extracts used by Polakis & Bartley (1965). The methods of assay are described in Table 1.

RESULTS

Development of mitochondria in galactose-grown cells. Plate 1 shows yeast cells grown on 0.9% galactose anaerobically in the complete medium at the early exponential phase of growth (13 hr. after inoculation). In contrast with cells grown on glucose (Polakis *et al.* 1964) the cells grown on galactose have both vacuolar structures and mitochondria. Cells fixed at later stages of growth all show mitochondria.

Plate 2 shows again yeast cells grown on 0.9% galactose aerobically in the complete medium at the early exponential phase of growth (12 hr. after inoculation). Most of the galactose (47 μ moles/ml.) is still present in the medium, but again in contrast with glucose-grown cells (Polakis *et al.* 1964) the galactose-grown cells have easily discernible mitochondria, as have cells fixed at subsequent stages of growth.

Development of mitochondria in aerobically grown yeast cells on very low glucose concentrations. Plate 3 shows yeast cells grown on 0.09% glucose in a complete medium 10 hr. after the inoculation, when very small amounts of glucose are still present in the medium (0.06 μ mole/ml.). Again in contrast with cells grown on 0.9% glucose (Polakis *et al.* 1964) and fixed at about the same stage of growth, the cells grown on very small glucose con-

EXPLANATION OF PLATE I

Electron-microscope picture of a section through yeast cells grown anaerobically on 0.9 % galactose for 13hr.

N, Nucleus; V, vacuolar structure; M, mitochondria. Magnification: $\times 14000$.

EXPLANATION OF PLATE 2

Electron-microscope picture of a section through yeast cells grown aerobically on 0.9% galactose for 12hr.

N, Nucleus; V, vacuolar structure; M, mitochondria. Magnification: $\times 14000$.

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EXPLANATION OF PLATE 3

Electron-microscope picture of sections through yeast cells grown aerobically on 0.09% glucose for 10 hr. N, Nucleus; V, vacuolar structure; M, mitochondria. Magnification: $\times 14000$.

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Table 1. *Measurement of enzyme activities*

Enzyme (a)	Buffer (b)	Other additions (c)	Substrate(s) (d)	Reference cuvette does not contain (e)	Reaction started by addition of (f)	Wavelength employed and molecular extinction coefficient ϵ ($M^{-1}cm^{-1}$) (g)	Reference (h)
NADH-cytochrome <i>c</i> oxido- reductase (EC 1.6.2.1)	Potassium phos- phate buffer, pH 6.45 (50 mM)	EDTA (0.33 mM); bovine plasma albumin (dialysed) (15 mg.); CN ⁻ (1.67 mM); cell-free extract	Cytochrome <i>c</i> (3 mg.); NADH (0.1 mM)	NADH	NADH	550 m μ 19.1×10^3	Green & Ziegler (1963)
NADPH-cytochrome <i>c</i> oxido- reductase (EC 1.6.2.3)	Potassium phos- phate buffer, pH 6.45 (50 mM)	EDTA (0.33 mM); bovine plasma albumin (dialysed) (15 mg.); CN ⁻ (1.67 mM); cell-free extract	Cytochrome <i>c</i> (3 mg.); NADPH (0.1 mM)	NADPH	NADPH	550 m μ 19.1×10^3	Adaptation of the assay for NADH- cytochrome <i>c</i> oxidoreductase Mackier, Collip, Duncan, Appaji- Rao & Huenne- kens (1962)
Succinate-cytochrome <i>c</i> oxido- reductase	Potassium phos- phate buffer, pH 6.45 (50 mM)	NaN ₃ (10 mM); cell-free extract	Succinate (10 mM); cytochrome <i>c</i> (3 mg.)	Succinate	Succinate	550 m μ 19.1×10^3	Green & Ziegler (1963)
NADH oxidase (NADH-oxygen oxidoreductase, EC 1.6.99)	Potassium phos- phate buffer, pH 6.45 (50 mM)	EDTA (0.33 mM); bovine plasma albumin (dialysed) (15 mg.); cell-free extract	NADH (0.1 mM)	NADH	NADH	340 m μ 6.22×10^3	Adaptation of the assay for NADH oxidase Polakis <i>et al.</i> (1964)
NADPH oxidase (NADPH- oxygen oxidoreductase)	Potassium phos- phate buffer, pH 6.45 (50 mM)	EDTA (0.33 mM); bovine plasma albumin (dialysed) (15 mg.); cell-free extract	NADPH (0.1 mM)	NADPH	NADPH	340 m μ 6.22×10^3	
Cytochrome <i>c</i> -oxygen oxido- reductase (EC 1.9.3.1)	Potassium phos- phate buffer, pH 6.45 (50 mM)	EDTA (0.33 mM); bovine plasma albumin (dialysed) (15 mg.); cell-free extract	Cytochrome <i>c</i> (reduced) (0.6 mg., 16 μ M)	Cytochrome <i>c</i>	Cell-free extract	550 m μ 19.1×10^3	

* Activities were determined both in the presence and absence of cytochrome *c*.

Table 2. *Changes in activities of oxidative enzymes during growth of yeast*

For details see the Materials and Methods section and Table 1.

Carbon source			Enzyme activity (μmoles/min./mg. of protein)												Pyruvate	
Growth medium			Glucose				Galactose				Synthetic				Complete	Synthetic
...	...	...	Complete	Synthetic			Complete	Synthetic			Complete	Synthetic			Complete	Synthetic
...	...	...	10 $\frac{1}{2}$	16	24	12	15	30	14	27	17	27	27	27	27	27
Time of growth (hr.)	...	...	200	220	Late	35	66	78	40	180	37	78	37	78	45	12
Turbidity of culture	...	...	Second stage, exponential growth	Second stage, exponential growth	Second stage, exponential growth	First stage, exponential growth	Second stage, exponential growth	Late second stage, exponential growth	First stage, exponential growth	Second stage, exponential growth	First stage, exponential growth	Second stage, exponential growth	First stage, exponential growth	Second stage, exponential growth	Late second stage, exponential growth	Late second stage, exponential growth
Phase of growth	...	...	(c)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)	(k)	(l)	(m)	(n)	(o)
Enzyme (a)	...	...	58.7	202	230	23.6	61.2	94.1	53.8	238	48.3	145	170	86.5	86.5	86.5
NADH-cytochrome c oxidoreductase	...	...	65.9	67.6	69.3	64.2	69.9	64.4	39.9	37.7	43.5	52.9	50.8	24.6	24.6	24.6
NADPH-cytochrome c oxidoreductase	...	...	6.5	56.2	56.2	1.3	13.0	21.2	10.0	54.8	6.5	31.0	51.7	32.0	32.0	32.0
Succinate-cytochrome c oxidoreductase	...	...	(12.3) 200.4	(65.1) 1062	(62.7) 1023	(2.4) 39.0	(15.3) 249.3	(20.4) 333	(30.3) 492	(67.2) 1095	(14.7) 240.0	(33.3) 543	(52.2) 852	(22.2) 363	(22.2) 363	(22.2) 363
Cytochrome c oxidase*	...	...	50.0 (31.4)	142 (50.8)	139 (48)	13.8 (7.5)	64.9 (44.5)	62.4 (34.5)	52.6 (29.3)	166 (88.1)	77.2	102 (73.0)	200 (110)	310 (147)	310 (147)	310 (147)
NADH oxidase†	...	...	24.5 (9.8)	37 (16.9)	35.9 (15.8)	27.6 (10.0)	30.6 (9.3)	32.3 (10.8)	13.0 (7.1)	21.0 (19.0)	15.7	14.1 (9.4)	17.9 (12.1)	20.8 (20.8)	20.8 (20.8)	20.8 (20.8)
NADPH oxidase†	...	...														

* Values in parentheses are the first-order velocity constants (min.^{-1}) calculated according to Smith (1955). The initial rate given is derived by multiplication of the constant by the concentration of cytochrome *c* employed.

† Values in parentheses are the activities of the enzymes measured without the addition of cytochrome *c*.

centrations clearly show mitochondria and vacuolar structures.

Activities of the respiratory enzymes. The changes in the activities of respiratory enzymes of yeast cells grown on different carbon sources, in different media and at different stages of growth, are given in Table 2. For the amounts of substrates consumed and ethanol produced at the different stages of growth see Table 2 of Polakis & Bartley (1965).

NADH- and NADPH-oxidizing enzymes. NADPH-oxidizing enzymes were not repressed by sugars. Both NADPH-cytochrome *c* oxidoreductase and NADPH oxidase were more active in glucose-grown cells. Schatz & Klima (1964) have shown that NADPH-oxidizing enzymes are associated with the microsomal fraction in yeast and their activity remains essentially the same in aerobically and anaerobically-grown cells of baker's yeast, as well as in cells of the 'petite' mutant. Our results are in agreement with the observations of these authors.

The other three enzymes (cytochromes *c* oxidase, NADH-cytochrome *c* oxidoreductase and NADH oxidase) were all repressed by sugars and especially by glucose (see also Polakis *et al.* 1964). The repression was more marked in the synthetic media. Thus in the first stage of growth the activities of cytochrome *c* oxidase and NADH oxidase of cells grown on glucose in a synthetic medium were 6-fold lower than the activities of the cells grown on galactose. This difference was about the same as for succinate-cytochrome *c* oxidoreductase.

In the second stage of growth an increase of the activities was observed (5-fold in glucose-grown cells but much less, 1.5–3-fold, in galactose-grown cells), but activities of cells grown in the complete media remained about twice as high as those of cells grown in the synthetic media. Again galactose-grown cells had higher activities.

The highest NADH-oxidase activity was recorded in cells growing on pyruvate.

Succinate-cytochrome c oxidoreductase. During the first stage of growth the activity of the enzyme system was negligible in cells grown on glucose. For example, measurement of the activity in cells grown in a complete medium and harvested when glucose was still present gave values of about 1–2 $\mu\text{moles/min./mg.}$ of protein. Galactose repressed the formation of the enzyme but less effectively than glucose, and generally activities of galactose-grown cells were about 6-fold higher than those of glucose-grown cells. At the second stage of growth the activity increased, especially in cells grown on glucose, and the activities in the complete medium became about twice as high as those in the synthetic one. The activity of the enzyme in cells grown on pyruvate was appreciable, but again cells grown in the synthetic medium had lower activities.

DISCUSSION

Tustanoff & Bartley (1964a) have shown that during the aerobic adaptation on glucose of yeast cells grown anaerobically on glucose the uptake of oxygen by the cells starts when the major part of the glucose has been consumed, and the same authors (Tustanoff & Bartley, 1964b) have also shown that, although cytochrome *c* oxidase virtually disappeared from yeast cells grown anaerobically on glucose, a substantial activity was present in cells grown anaerobically on galactose and also that cells grown on galactose anaerobically could immediately respire on glucose, in contrast with cells grown on glucose. The observations of these authors, as well as the evidence presented above, give further support to the suggestion made by Polakis *et al.* (1964) that the respiratory adaptation of yeast is not an induction by oxygen but a de-repression due to the removal of glucose, and also that the development of mitochondria is due to de-repression after the consumption of glucose rather than to an induction by oxygen. These observations lead to the question: what controls the formation of mitochondria?

Although no proof can be given, there are now some observations suggesting that the repression of formation of mitochondrial structure and the mitochondrial enzymes by glucose is caused by the ready availability of 'high-energy' substances from a source other than oxidative phosphorylation (ATP or any other 'high-energy' compound readily produced from glucose) and is a mechanism similar to an end-product inhibition. These observations are:

(a) The activities of the enzymes of the citric acid cycle (Polakis & Bartley, 1965) and of the respiratory chain in cells grown on sugars during the first stage of growth are repressed more by glucose than by galactose, and the highest repression is observed with the respiratory enzyme systems succinate-cytochrome *c* oxidoreductase, NADH oxidase and cytochrome *c* oxidase. Thus in cells grown in the synthetic medium the activities of these enzyme systems are 6-fold higher when galactose is the carbon source (Table 2).

(b) Galactose- and glucose-grown cells have been found to grow aerobically at the same rate in the exponential phase of growth, although galactose disappears more slowly from the medium. Thus the energy that the glucose-grown cells have obtained by glycolysis has to be obtained in galactose-grown cells by some other pathway, presumably the citric acid cycle and the respiratory chain. The differences in the amount of ethanol produced by cells growing on either glucose or galactose, during the first stage of growth (Polakis & Bartley, 1965), confirm this observation.

(c) Yeast cells, although growing at the same rate on both sugars aerobically, clearly grow more slowly on galactose anaerobically (Tustanoff & Bartley, 1964b).

(d) Since low activities of the enzymes found in glucose-grown cells suffice to provide intermediates for growth at a rate equal to the rate of growth on galactose, some of the extra activities of these enzymes found in galactose-grown cells are probably used for the supply of energy rather than intermediates. Since the activity of α -oxoglutarate oxidase in the glucose-grown cells is only one-fifth of that in galactose-grown cells, there will be a higher concentration of α -oxoglutarate in the first stage of growth on glucose, thus facilitating glutamate synthesis. The higher concentration of α -oxoglutarate oxidase in galactose-grown cells suggests that the citric acid cycle is used for both biosynthetic and degradative processes.

All these observations could be interpreted by assuming that, during the first stage of growth of yeast cells on sugars, while the citric acid cycle is used by glucose-grown cells only as a biosynthetic pathway for the supply of intermediates, in galactose-grown cells it is used both as a biosynthetic and as an energy supplying pathway. Glucose-grown cells obtained the energy needed for growth exclusively through glycolysis because the rate of glycolysis is sufficiently fast, and as a consequence glucose represses the other pathway of energy production, i.e. the respiratory chain, and especially the coupling of the oxidation of NADH or succinate to the production of energy. This in turn results in the observed repression of mitochondrial formation.

In contrast, galactose-grown cells, being in need of greater amounts of energy to attain the maximum rate of growth, require a supplementation of the energy obtained by glycolysis and this results in the partial de-repression of the enzymes of the citric acid cycle, and especially of the respiratory chain. It is possibly the development of these higher activities of the enzymes, and especially the coupling of the respiratory chain to the production of energy, that requires the development of true mitochondrial structures.

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