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Control of growth yield of yeast on respiratory substrate by mitochondrial content

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Abstract

It is well documented that the growth yield of microorganisms depends on the fraction of ATP utilized for cell maintenance compared to that used for biomass synthesis per se. During aerobic growth, the growth yield may also be a function of the yield of ATP synthesis by oxidative phosphorylation (i.e. ATP/O ratio), a parameter which can vary in vitro according to the functional steady state of mitochondria.

In this respect, the enthalpic growth yield of yeast aerobic cultures has been assessed by direct microcalorimetry during the transition from exponential growth to stationary phase. Under these conditions, the ATP turnover largely decreased whereas, the growth yield remained quite constant. This steady yield was due to a decrease in the mitochondrial content throughout the transition period.

The Ras/cAMP/PKA signaling pathway is known to regulate adaptations of yeast cells upon environmental and metabolic transitions. We further analyzed yeast mutants overactivated for this pathway under fully aerobic growth conditions. Overactivation induced a proliferation of mitochondria within the cells which was correlated with a high respiratory activity and a drop in the enthalpic growth yield. Taken together, these results indicate that mitochondria by themselves can behave as energy dissipative systems under conditions where the oxidative phosphorylation capacity largely exceeds the ATP needs for cell growth. These data emphasize the physiological importance of the regulation of mitochondrial biogenesis in the optimization of the growth yield versus growth rate.

Keywords: Yeast; Microcalorimetry; Enthalpic growth yield; Mitochondria; Oxidative phosphorylation; cAMP

1. Introduction

Microbial growth is a striking adaptation of microorganisms to environmental changes to reach a compromise between growth rate, growth yield and thermodynamic growth efficiency. For decades, quantitative and theoretical tools have been developed to describe such phenomena. For instance, Metabolic Control Analysis has been widely used to quantify the kinetic control of the growth rate that may arise during variations of the enzymatic equipment of growing cells (e.g. see [1–3] for the F_1F_0-type H^+ ATP synthase, P-type H^+ ATPase and bc1-respiratory chain

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complex, respectively) or during nutrient-limited growth [4]. Moreover, by establishing the balanced chemical reactions for anabolism and catabolism, it has been possible to determine mass yield and carbon yield (YX/S in C mol of biomass formed per C mol of substrate used) of the growth process [5]. Taking the reference reaction of substrates and products of the growth as a combustion, the previous approach was further simplified by the determination of the enthalpic growth yield, obtained from the continuous measurement of the heat yield by direct microcalorimetry [6,7]. Growth yield has been shown to depend on: (i) strain and carbon source metabolism based on the enzymatic equipment; (ii) futile cycling, uncoupling and overflow metabolism (by-product formation); and (iii) ATP requirements for cellular maintenance. Finally, by considering growing cells as open energy transformers, microbial growth optimization has been quantitatively analyzed by applying non-equilibrium thermodynamics based on the kinetic (e.g. growth rate) and thermodynamic properties of the system (e.g. Gibbs energy changes related to substrates and products of the overall growth reaction, including biomass) [8]. Using this approach and by varying the carbon source, it has been possible to understand the interdependence between the growth rate, growth yield and thermodynamic work efficiency. In fact, microbial growth optimization has been interpreted in terms of maximal growth rate and maximal growth yield when the substrate was highly reduced, resulting in a very low efficiency (e.g. η reaches -0.35 with ethanol because the ΔG of anabolic reactions is negative), or in terms of maximal growth rate and low growth yield at high efficiency (e.g. η reaches $+0.24$ with oxalate) when the substrate was highly oxidized [9,10].

The strategy of optimizing growth rate and yield has been mainly studied under conditions of active exponential growth. In this paper, we analyze the variation in enthalpic growth yield under conditions where a long-term adaptation of the yeast *Saccharomyces cerevisiae* occurs in response to carbon source depletion in the culture medium. We particularly focus on the role played by the mitochondrial compartment during the transition from exponential growth to stationary phase, during aerobic metabolism. Hence, during this transition, as the ATP-demand for growth decreases, the cellular mitochondrial content is down-modulated, which in turn causes a decrease in the respiratory activity and leads to a steady enthalpic growth yield. Moreover, since the Ras/cAMP/protein kinase A signaling pathway has been shown to be involved in metabolic transitions and stress responses, we have investigated the effect of an over-activation of this pathway on the growth characteristics of yeast cells and on the development of the mitochondrial compartment. The major contribution of this work is that the cAMP signaling pathway has been found to up-regulate the mitochondrial content during the aerobic growth of yeast, which in turn diminishes the optimization of growth yield versus growth rate.

2. Materials and methods

2.1. Yeast strains, culture medium and growth condition

The *S. cerevisiae* strains used were the haploid W303-1a strain (*ade*2-10, *his*3-11, 112, *trp*1-1, *ura*3-1, *can*100°) and the diploid OL556 strain (*α/α*, *cdc25-5/cdc25-5*, *his3/his3*, *leu2/leu2*, *rca1(pde2)/rca1*, *TRP1/trp1*, *ura3/ura3*) supplied by M. Jacquet (Orsay, France). Cells were grown aerobically at 28 °C on the following minimal medium: 0.17% yeast nitrogen base w/o amino-acids w/o ammonium sulfate (Difco), 0.1% potassium phosphate, 0.5% ammonium sulfate, pH 5.5, containing either 0.2 or 2% (w/v) of D, L-lactate (Aldrich). The concentration of auxotrophic requirements was 100 mg/l, except for leucine (800 mg/l) and histidine (300 mg/l). For the OL556 strain, the culture medium was supplemented with 0.1% casamino acids (Merck).

2.2. Growth determination

Growth was followed turbidimetrically at 600 nm in a Phillips spectrophotometer. Dry weight determinations were performed on samples of cells harvested throughout the exponential growth period and washed twice in distilled water. A coefficient of turbidity was obtained for OL556 cells grown in the absence and in the presence of 3 mM cAMP (0.19 ± 0.01 and 0.17 ± 0.02 mg dry weight/o.d. unit, respectively) and for W303-1a cells (0.17 ± 0.01 mg dry

weight/o.d. unit, regardless of the growth phase). Enthalpy of combustion of biomass was calculated from the determination of elemental composition of biomass ($CH_{1.96}O_{0.6}N_{0.19}$ for W303-1a, $CH_{1.89}O_{0.55}N_{0.2}$ and $CH_{1.91}O_{0.59}N_{0.16}$ for OL556 grown in the presence or not of cAMP, respectively) which was performed at the Ecole Nationale Supérieure de Chimie de Toulouse (Toulouse, France).

2.3. Calorimetric set-up and heat measurement assay

The heat production rate (i.e. $P = dQ/dt$, expressed as μW/ml) was continuously monitored at sampling intervals of 10 s with a multichannel microcalorimeter (thermal activity monitor (TAM), Thermometric AB, Jarfalla, Sweden) in the flow-through mode. The actual volume of the flow cell was measured using the triacetin calibration procedure [11] and equals 0.6 ml. Calibration and correction of the data were done by the Digitam software.

Cells were incubated in a water bath at 28 °C and were stirred by a magnetic stirrer at 300–400 rpm. Using a peristaltic pump (Gilson), they were transported via teflon tubing to the calorimeter (thermostated at 28 °C) and back to the flask. The flow rate of the pump was 2–3.5 ml/min, resulting in a total transport time of 0.5–1 min to avoid hypoxia. Cell cultures (200 ml) were performed under constant bubbling of sterile humidified air in 1 l flasks. Throughout the culture time, the cell suspension was sampled to measure optical density, metabolites in the culture medium and oxygen consumption rate.

2.4. Respiration assay

The oxygen consumption of cells and mitochondria was measured polarographically at 28 °C using a Clark oxygen electrode in a 2 ml thermostatically controlled chamber (Oroboros Oxygraph, Paar, Graz, Austria). Data were recorded at sampling intervals of 1 s (DatLab Acquisition software, Oroboros, Innsbruck, Austria). For respiration assays, 2 ml of growing cell suspension were quickly transferred to the respirometer cuve from the flask connected to the microcalorimeter. Respiratory rates (J_o) were determined from the slope of a plot of O_2 concentration versus time, divided by the biomass concentration.

2.5. Preparation of mitochondria, respiration and ATP synthesis assay

Mitochondria were isolated from protoplasts as in [12]. Protein concentration was measured by the biuret method using the bovine serum albumin as standard. Respiration and phosphorylation rates were measured as described in [13], in the presence of 4 mM NADH as respiratory substrate, 1 mM ADP and different concentrations of Pi in the incubation medium.

2.6. Determination of metabolite contents of culture media

Samples of growing cells in suspension were filtered prior to measuring the metabolite content in the culture medium. Pyruvate was measured enzymatically as described in [14]. D, L-lactate and acetate were measured by using enzyme combination kits (Biochemica Test Combination; Boehringer Mannheim).

2.7. Electron microscopy

Yeast cells were centrifuged for 1 min at 2000 × *g* and the pellets were cryofixated by quick submersion in liquid propane pre-cooled to −180 °C by liquid nitrogen. The loops were then transferred to a pre-cooled solution of 4% osmium tetroxide in dry acetone at −82 °C for 2 days and warmed gradually to room temperature before three washes in dry acetone. Specimens were then stained for 1 h in 2% uranyl acetate in acetone at 4 °C (darkroom). After another rinse in dry acetone, loops were infiltrated with araldite and ultrathin sections were stained with lead citrate before observation at 80 kV in a Philips EM10 electron microscope.

3. Results and discussion

3.1. Growth yield and oxidative phosphorylation during fully aerobic metabolism of yeast cells

In living cells, growth is the result of coupling between substrate catabolism and multiple anabolic reactions taking place during net biomass synthesis. In this coupling, ATP cycling plays a central role. Moreover, when eucaryotic cells are aerobically grown

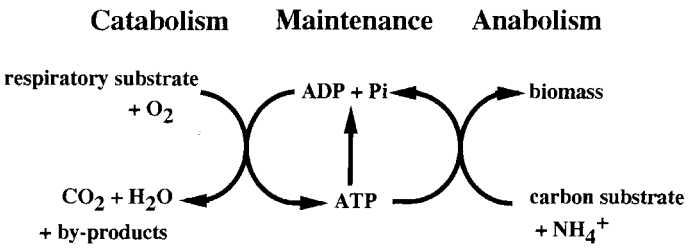

Fig. 1. Aerobic growth process of *S. cerevisiae*. For simplicity, energy transformations are only exemplified by catabolism, net anabolism and maintenance, assuming that all energy-transforming intermediates are kept at steady state (e.g. a complete ATP turnover occurs at constant concentration of ATP, ADP, Pi and constant transmembrane electrochemical potential differences). Maintenance reactions are defined to keep cell homeostasis steady (the initial state equals the final state). The total enthalpy change linked to the growth is the sum of the enthalpy changes of the anabolic reaction and of the catabolic reaction. It varies according to the growth yield. Assuming the reference state to be combustion products (CO_2 and H_2O), the enthalpic growth yield is equal to: $Y_h = (\Delta_c Q_x/(\Delta_r Q_x + \Delta_c Q_x) \times 100$, where $\Delta_r Q_x$ is the heat production measured during the synthesis of 1 g of biomass and $\Delta_c Q_x$, the heat obtained from the combustion of 1 g of biomass.

with a non-fermentable substrate as sole carbon and energy source, ATP synthesis mainly relies on electron transport phosphorylation in mitochondria, as compared to substrate level phosphorylation.

In this global scheme, the growth yield represents the amount of carbon substrate assimilated into biomass as compared to the total amount of substrate utilized for all metabolic processes (Fig. 1). Under steady state conditions where ATP synthesis matches ATP consumption, the growth yield may depend on two variables.

First, the fraction of ATP utilized for cell maintenance (e.g. ionic gradient, turnover of cell components) compared to that used for biomass synthesis per se. Unfortunately, energy requiring metabolic processes are not easily attributed to either maintenance or anabolic non-maintenance processes. Nevertheless, in several studies carried out on yeasts, the maintenance energy requirements were found to be low. However, under conditions of external stress (e.g. ionic and osmotic stress; extracellular pH stress; weak acid-induced intracellular pH stress), the maintenance may increase considerably [15,16].

Second, the growth yield may also be a function of the amount of ATP synthesized per substrate catabolized, i.e. the amount of ATP synthesized per oxygen consumed during electron transport phosphorylation (i.e. ATP/O ratio) (Fig. 1). Indeed, according to the chemiosmotic coupling of oxidative phosphorylation, the proton electrochemical difference across the mitochondrial inner membrane acts as a coupling intermediate between ATP synthase and respiratory chain (Fig. 2A). As shown in Fig. 2B, the yield of oxidative phosphorylation is the amount of ATP produced per total oxygen consumed at any steady state of respiration driven by proton leak and ATP synthesis. The actual ATP/O ratio has been shown in vitro to vary according to the functional steady state of mitochondria; it varies from zero under non-phosphorylating conditions (state 4) to the maximal ATP/O ratio that can be sustained in the presence of saturating amounts of Pi, ADP and respiratory substrate (state 3) (Fig.2A). Thus, by extrapolating these results to the in vivo situation, the growth yield should be lowered when the ATP turnover decreases, because the consumption of the respiratory substrate required to compensate H^+ leak and H^+ pump slipping increases.

3.2. Evolution of enthalpic growth yield during transition from exponential growth to stationary phase

The heat dissipation associated with the growth of *S. cerevisiae* in a medium containing a limiting amount of a non-fermentable carbon substrate, i.e. D, L-lactate, was continuously monitored by direct microcalorimetry. In the typical experiment in Fig. 3A, three distinct phases can be defined: in the exponential phase, the heat production rate increased in parallel with biomass production and lactate consumption; in the transition phase occurring when about one third of the lactate was consumed, the heat dissipation pattern no longer correlated with the biomass increase (Fig. 3A); in the stationary phase, the carbon source was exhausted and the heat production rate

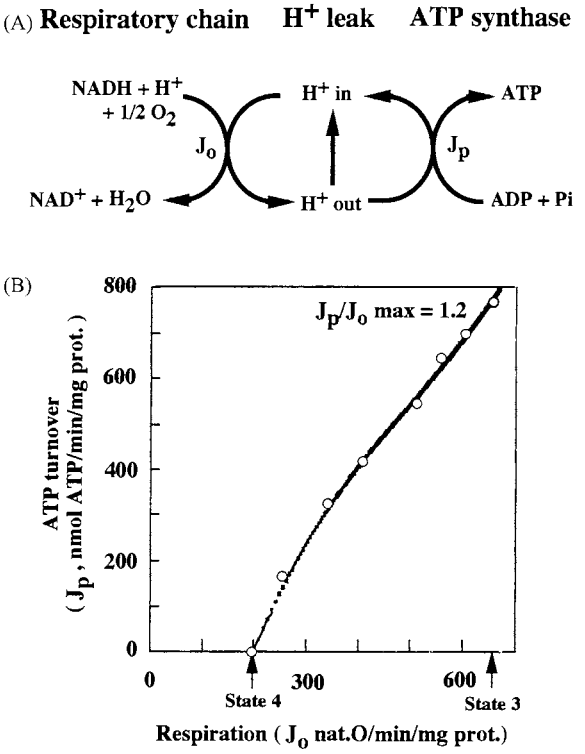

Fig. 2. Mitochondrial oxidative phosphorylation. Oxidative phosphorylation results in a chemiosmotic coupling between F_1F_0 ATP synthase and the proton pumps of the respiratory chain. At any steady mitochondrial state (e.g. constant proton electrochemical difference across the inner membrane, constant ATP synthesis et respiratory rates), the proton efflux by the respiratory chain compensates the proton leak and the proton influx through ATP synthase. The insert represents the ATP synthesis rate as a function of the respiratory rate during Pi titration of oxidative phosphorylation of isolated yeast mitochondria, and in the presence of saturating amounts of ADP and NADH as respiratory substrate. State 4 is the non-phosphorylating respiration which compensates the proton leak and proton pump slipping. State 3 is the maximal phosphorylating steady state (i.e. at saturation of Pi and ADP) that gives the maximal yield (i.e. ATP synthesis-oxygen consumption flux ratio).

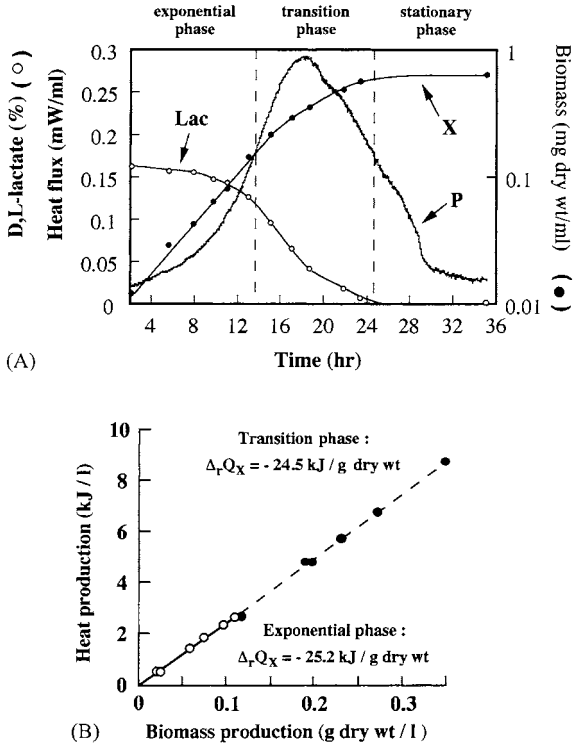

Fig. 3. Transition from exponential phase to stationary phase during the growth of W303-1a yeast in bath culture with lactate. W303-1a cells were grown aerobically in minimal medium containing 0.2% (w/v) D, L-lactate. (A) Heat production (P) (continuous line), D, L-lactate concentration in the culture medium (Lac) (○) and biomass (X) (●) were measured as described in Section 2 (typical experiment); (B) integrated heat dissipated as a function of the cumulative biomass production, in the exponential (○) and transition phase (●). The heat yield in these phases ($\Delta_r Q_X$) was calculated from the respective slope values.

finally reached a constant and low value, in agreement with the growth arrest of yeast cells (Fig. 3A). Accumulation of the major by-products of the lactate catabolism was also measured in the culture medium throughout the culture period. For instance, pyruvate and acetate concentrations slowly increased during the exponential phase to reach concentrations of about 2 and 1 mM, respectively. Pyruvate was still produced in the transition phase, whereas acetate was consumed. In the stationary phase, a residual pyruvate concentration was still detectable in the culture medium (data not shown). Fig. 3B represents integrated heat dissipation (calculated from Fig. 3A) as a function of the cumulative biomass production during both the exponential and transition growth phases. These plots gave a linear correlation in both phases, so they could be considered as constant physiological states. Interestingly, the heat yield ($\Delta_r Q_X$), calculated from the slopes of Fig. 3B was not significantly different in the exponential and transition growth phases (−25.2 versus −24.5 kJ/g dry weight). Taking into account the enthalpy of the combustion of biomass ($\Delta_c Q_X$) of -19.2 ± 0.4 kJ/g dry weight,

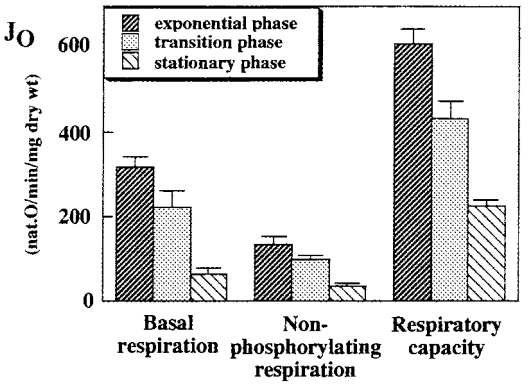

Fig. 4. Respiratory activity of W303-1a yeast cells in the different growth phases. W303-1a cells were grown aerobically as described in Fig. 3A. Cell samples were taken from the culture flask in the exponential phase (2–4 h), in the mid-transition phase (18–22 h) and in the stationary phase (30–34 h). Respiratory rate of yeast cells was measured either in the growth medium without any addition (basal), in the presence of the F_1F_0 ATP synthase inhibitor, triethyltin (non-phosphorylating respiration), or in the presence of a protonophoric uncoupler (respiratory capacity) as described in [17].

the enthalpic growth yield has been calculated according to the principle of enthalpy balance as: $Y_h = \Delta_c Q_x/(\Delta_r Q_x + \Delta_c Q_x) \times 100$ [7,17]. It is worth noting that the enthalpic growth yield was nearly the same in the exponential (43%) and transition phases (44%).

We have further analyzed the respiratory activity of yeast cells throughout the culture period. Fig. 4 shows that the basal respiratory activity measured directly in the culture medium decreased during the transition from exponential to stationary phase, thus indicating that the ATP-turnover was decreased in response to growth arrest. To estimate the part of oxygen uptake coupled to ATP synthesis in these phases, we used the cell-permeant specific inhibitor of the F_0F_1 ATP synthase, triethyltin (TET) [18]. In this case, the TET-insensitive oxygen uptake represents the respiratory activity not associated with mitochondrial ATP synthesis. Like the basal oxygen uptake, TET-insensitive oxygen uptake of cells decreased during the transition phase and reached a minimal value in the stationary phase. This implies that the respiratory activity and, therefore, the carbon substrate consumption driven by the proton leak and redox pump slipping decreased during the transition period. Moreover, the respiratory capacity of yeast cells was measured by means of the protonophoric uncoupler, CCCP, which dissipates the proton electrochemical difference across the inner mitochondrial membrane, and thereby stimulates the respiratory rate to its maximum value [17]. Fig. 4 shows that the maximal respiratory capacity also decreased during the transition from exponential to stationary phase. This phenomenon has been further correlated with a decrease in the mitochondrial enzyme content (e.g. cytochromes, D, L-lactate dehydrogenases, citrate synthase) [19].

In conclusion, during a purely respiratory growth, a modulation of the mitochondrial content seems to be involved in a long-term regulation of oxidative phosphorylation. Our results indicate that the respiration driven by the proton leak and proton pump slipping is the major energy dissipative system in a fully aerobic metabolism. When the ATP-turnover decreases in response to growth arrest, this energy dissipation can be regulated long-term by a decrease in the mitochondrial content. This adaptation leads to a constant enthalpic growth yield during the final generation of the culture. The questions raised by our findings are the following: (i) what are the mechanisms underlying the decrease in the mitochondrial content: an activation of proteolytic activity and/or an inhibition of the mitochondria biogenesis? and (ii) what are the signals leading to this down-regulation? During aerobic growth, a modulation of the amount of mitochondrial seems to be involved in a long-term regulation of oxidative phosphorylation. In this respect, the ccs1-1 yeast strain, which is mutated in the *IRA*2 gene encoding for an activating protein of the Ras-GTPase activity, was characterized by a higher cellular respiration in parallel with a higher cytochrome content in the late exponential phase [20–22]. Moreover, this strain exhibited a number of phenotypic characteristics of cells over-activated for the Ras/cAMP/protein kinase A pathway, i.e. a nutrient starvation sensitivity in the stationary phase and a reduced glycogen accumulation. Hence, our previous observations suggested that the Ras/cAMP/cAPK pathway was involved in the regulation of the mitochondrial content of cells in the transition phase. We therefore examined the effect of an overactivation of the cAMP signaling pathway on the development of the mitochondrial compartment and on the growth yield of yeast mutants grown in the presence of a respiratory substrate.

3.3. Modulation of mitochondrial content, growth rate and growth yield by cAMP signaling pathway

In the OL556 yeast strain, it has been previously documented that the intracellular level of cAMP can be modulated. Hence, at a permissive temperature of 28 °C, the cdc25-5 mutation produced a low but sufficient level of cAMP to allow cell division, while the *rca*1 mutated allele of the *PDE*2 gene caused a defect in the high affinity phosphodiesterase and allowed the manipulation of intracellular cAMP levels by adding cAMP to the culture medium [23,24]. The effects of cAMP addition on growth rate, enthalpic growth yield and cellular respiration were analyzed on OL556 cells batch grown on lactate 2 and 0.2%.

With 2% lactate as carbon source, OL556 cells were able to grow in the presence of 3 mM cAMP without significant change in their doubling time (i.e. 3.5 ± 0.25 h regardless of the presence or not of cAMP in the culture medium). For each condition, we measured the basal respiratory activity, the non-phosphorylating respiration (i.e. in the presence of TET) and the respiratory capacity (i.e. in the presence of CCCP). The basal respiration was 1.8-fold stimulated when cAMP was present in the culture medium. The TET-insensitive oxygen consumption and the fully uncoupled respiration were also 1.6- and 1.8-fold increased, respectively by cAMP addition (Fig. 5). This parallel increase in endogenous, TET-insensitive and fully uncoupled respiratory rates suggested that a modulation of the mitochondrial equipment occurred in response to an over-activation of the cAMP-protein kinase A pathway. To investigate whether this cAMP-induced increase in respiration was associated with functional and/or morphological alteration of mitochondria within the cells, thin sections of yeast cells were observed by transmission electron microscopy. Fig. 6 shows that cAMP treatment induced an obvious proliferation of ultrastructurally well defined mitochondria. This mitochondrial proliferation was associated with a two-fold increase in both the total mitochondrial protein content and the mitochondrial cytochrome content and F_1F_0 ATPase, cytochrome oxidase and D + L lactate dehydrogenase activity [19]. Moreover, in vitro experiments performed on isolated mitochondria demonstrated that cAMP treatment of cells did not significantly change the coupling between respiration and ATP synthesis rate [19].

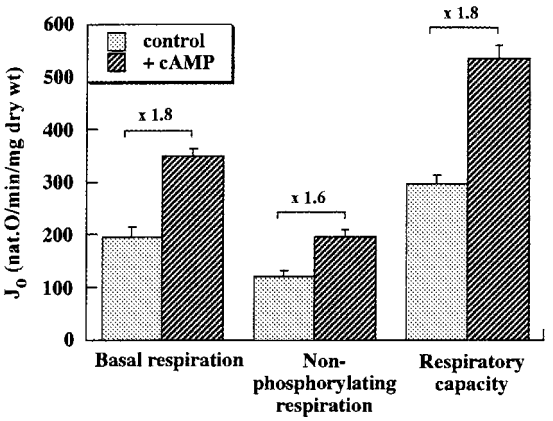

Fig. 5. Effect of cAMP treatment on respiratory activity of OL556 yeast. OL556 cells were grown aerobically with 2% lactate as carbon source, in the presence (black bars) or not (white bars) of 3 mM cAMP. For each condition, basal, non-phosphorylating and respiratory capacity of yeast cells were measured as described in Fig. 4. Values are means ± S.D. of three independent experiments.

Under the same culture conditions, we measured the heat flux of OL556 cells grown in the exponential phase in the presence or not of cAMP in the culture medium. Fig. 7 represents a typical experiment where the integrated heat dissipation was measured as a function of the biomass produced during the same time period. cAMP addition in the culture medium largely increased the heat produced during a given biomass synthesis. After repeating this experiment several times, we observed that the heat yield was about two-fold higher during the growth of OL556 cells in the presence of cAMP ($\Delta_r H_x = -38.5$ and -19 kJ/g dry wt., in the presence and absence of cAMP, respectively). Taking the respective values of the enthalpy of combustion of biomass, we observed that the calculated enthalpic growth yield (Y_h) largely decreased from 55 ± 3 to 34 ± 3% during cAMP treatment.

The same analysis was performed under conditions where the growth rate was kinetically limited by the lactate concentration in the batch culture. When cells were grown with 0.2% lactate, cAMP addition decreased the generation time by a factor of about 1.4 (i.e. 3.5 ± 0.25 and 4.7 ± 0.3 h in the presence or not of cAMP, respectively). However, the effect of cAMP on mitochondrial biogenesis was the same regardless of the lactate concentration in the culture medium, i.e.

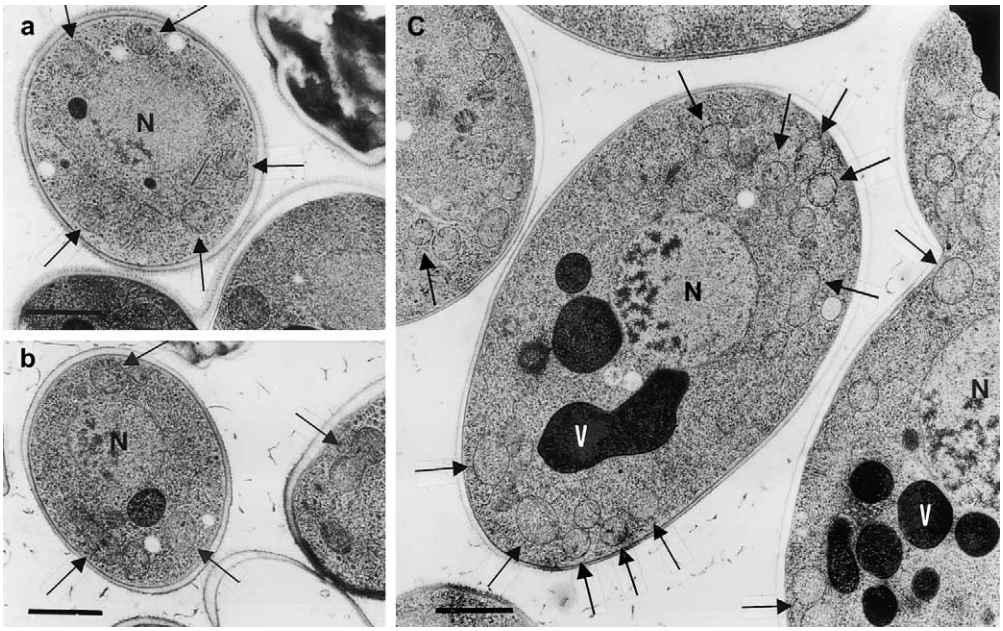

Fig. 6. Ultrastructural effect of cAMP on OL556 strain aerobically grown on lactate. OL556 cells were grown aerobically with 2% lactate as carbon source, in the presence or not of 3 mM cAMP, and were cryofixated and contrasted as described in Section 2. Transmission electron microscopy was then performed on ultrathin sections of control cells (a, b) and cAMP-treated cells (c). Mitochondria are indicated by arrows. Scale bar represents 1 μm.

an increase in the mitochondrial enzyme equipment, which was responsible for a 70% increase in both the basal respiration and in the maximal respiratory capacity of cells (data not shown). Interestingly, the enthalpic growth yield measured in the presence of 0.2% lactate was only slightly decreased by cAMP treatment (i.e. 44 ± 5 in the presence of cAMP compared to 55 ± 4 for control cells). This situation contrasts with that observed for 2% lactate batch cultures. In fact, in the presence of 0.2% lactate, up-regulation of the mitochondrial biogenesis by cAMP removed some of the kinetic constraints on biomass synthesis and therefore improved the growth rate, with a minor variation in the growth yield. In contrast, in the presence of 2% lactate, the growth rate was optimal and the increase in the mitochondrial content behaved strictly as an energy dissipative system, thus decreasing the growth yield.

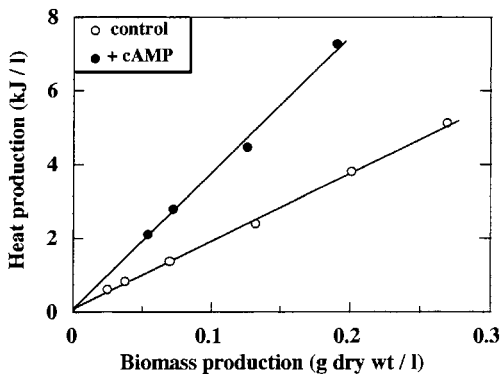

Fig. 7. Effect of cAMP on heat yield of OL556 strain grown on lactate. OL556 cells were grown aerobically with 2% lactate as carbon source, and the heat flux was measured on-line by direct microcalorimetry. During the same time period, the integrated heat production and the cumulative biomass synthesis were determined.

In conclusion, our data obtained on growing yeast cells fully capable of oxidative phosphorylation show that the optimization of the growth rate versus the growth yield is at least controlled by the mitochondrial content. The role of mitochondria in such a phenomenon could be due to the mechanism of coupling of oxidative phosphorylation. Indeed, within the chemiosmotic framework, the cellular respiration driven

by the proton leak represents a maintenance process operating at the expense of the growth yield. This emphasizes the physiological role of the regulation of mitochondrial biogenesis. In fact, Ras/cAMP/protein kinase signaling and other signaling pathways have been found to tightly modulate mitochondrial respiration and biogenesis in various eucaryotic cell types (for reviews: see Refs. [25–27]).

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