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Partitioning of electron flux between the respiratory chains of the yeast *Candida parapsilosis*: parallel working of the the two chains

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Partitioning of the electron flux between the classical and the alternative respiratory chains of the yeast *Candida parapsilosis*, was measured as a function of the oxidation rate and of the Q-pool redox poise. At low respiration rate, electrons from external NADH travelled preferentially through the alternative pathway as indicated by the antimycin A-insensitivity of electron flow. Inhibition of the alternative pathway by SHAM restored full antimycin A-sensitivity to the remaining electron flow. The dependence of the respiratory rate on the redox poise of the quinone pool was investigated when the electron flux was mediated either by the main respiratory chain (growth in the absence of antimycin A) or by the second respiratory chain (growth in the presence of antimycin A). In the former case, a linear relationship was found between these two parameters. In contrast, in the latter case, the relationship between Q-pool reduction level and electron flux was non-linear, but it could be resolved into two distinct curves. This second quinone is not reducible in the presence of antimycin A but only in the presence of high concentrations of myxothiazol or cyanide. Since two quinone species exist in *C. parapsilosis*, UQ_9 and Q_x ($C_{33}H_{54}O_4$), we hypothesized that these two curves could correspond to the functioning of the second quinone engaged during the alternative pathway activity. Partitioning of electrons between both respiratory chains could occur upstream of complex III with the second chain functioning in parallel to the main one, and with the additional possibility of merging into the main one at the complex IV level.

Introduction

The yeast *Candida parapsilosis* exhibits peculiar features at the bioenergetic level since its mitochondria possess two respiratory chains, the classical one and a second oxidative pathway (for review see Ref. 1) which differs from the well-documented alternative chains found in many plants and microorganisms (for reviews see Refs. 2, 3). In contrast to these pathways which branch off from the main respiratory chain at the ubiquinone level, it was hypothesized that the second respiratory chain of *C. parapsilosis*, which is antimycin A-insensitive but inhibited by amytal [4], SHAM and high concentrations of myxothiazol [5] or cyanide [1], parallels the classical respiratory chain with the possibility for electrons to merge into the main chain after the bc_1 complex [6,7]. Previously it has been demonstrated that exogenous substrates such as NADH, NADPH and glycerol 3-phosphate were preferentially oxidized by this second respiratory chain, and it was postulated that this chain was a functional replacement for the fermentative pathway, which is lacking in this yeast [1,8], and also allowed it to grow on a non-fermentable carbon source in the presence of drugs that inhibit the mitochondrial electron flow [9].

To date, some of the components of this second respiratory pathway have been characterized or/and isolated: (i) two dehydrogenases specific for NADH and NADPH oxidation respectively, located on the outer face of the inner membrane. These dehydrogenases are different from NDH-2 type rotenone-insensitive NADH-Q oxidoreductase of *Arum maculatum*, *Saccharomyces cerevisiae* or bacteria [10,11], and they have been characterized by their sensitivity to numerous inhibitors (amytal, mersalyl, butanedione) and their allosteric behaviour [4]; (ii) two cytochromes *c* have been isolated, which can be distinguished by their electron donor, their amino acid composition and their redox potential [6,7]. It was postulated that the existence of two cytochromes *c*, having midpoint redox potentials of 180 and 280 mV respectively, allowed electrons to be transferred to the cytochrome *c* oxidase or to the alternative oxidase; (iii) an alternative oxidase, sensitive to hydroxamates and high cyanide concentrations was detected spectrophotometrically by its absorption at 590 nm [6].

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It has also been recently demonstrated that mitochondria from *C. parapsilosis* possess low amounts of a quinone that is different from UQ_6 and UQ_9 [5]. Titrations of NADH oxidation with myxothiazol have revealed the presence of two classes of myxothiazol-sensitive binding sites, suggesting that two independent Q-binding proteins may be involved in each of the respiratory chains [5]. However, in most of the electron transfer chains studied up to now, ubiquinone appears as an obligatory intermediate carrier between the membrane-bound dehydrogenases (internal or external) and the cytochrome chain, and in plant mitochondria [12] as in fungi [13,14], it was assumed that alternative pathway branches off the main one at the ubiquinone level.

In *C. parapsilosis*, the presence of two classes of myxothyazol binding sites and of a peculiar quinone suggested a different regulation in the partitioning of electron fluxes between both respiratory pathways. Since the second quinone allows electrons to by-pass complex III, it was hypothesized that this partitioning between both pathways would not be a simple dependence on the redox poise of the quinone pool, as demonstrated to be the case in plant mitochondria [15–17].

In this study, we attempt to determine the partitioning of electron flux between both respiratory chains as a function of the respiratory rate and of the redox state of the quinone pool in *C. parapsilosis* when the electron flux is mediated either by the main respiratory chain (mitochondria from cells grown on glycerol) or through the second respiratory chain (mitochondria from cells grown on glycerol in the presence of antimycin A).

Materials and Methods

Preparation of mitochondria. Candida parapsilosis (CBS 7154) [18] was grown aerobically at 28°C in a complete culture medium supplemented with 2% glycerol as carbon source, with 2 mg/l of antimycin A added or not. Cells were harvested in the mid-exponential phase and mitochondria were isolated using the enzymatic procedure [19]. Protein concentration was measured by the biuret method using bovine serum albumin as a standard.

Respiration assay. Oxygen consumption was measured polarographically at 28°C in 2 ml of the following basal buffer: 0.65 M mannitol, 10 mM Tris maleate, 0.36 mM EGTA, 0.3% BSA (pH 6.7). The substrate was 5 mM succinate (1 mM ATP was added to activate succinate dehydrogenase [20] or a NADH-regenerating system: 4 mM glucose 6-phosphate, 2 mM NAD^+ and various amounts of glucose-6-phosphate dehydrogenase (EC 1.1.1.49) from *Leuconostoc mesenteroides* which is able to work with NAD^+ as cofactor [12].

Cytochrome spectra. These were recorded at room temperature using a double-beam dual-wavelength Aminco Chance DW2000 spectrophotometer.

Determination of the quinone reduction level. The redox state of exogenously added (1 mM) Q_2 was measured voltametrically in a specially constructed cell (University of Sussex Workshops) having a Rank oxygen electrode, a glassy carbon and platinium electrode, as previously described [15–17].

Characterization of the quinone. Quinone was isolated as previously described [5]; molecular weight and global composition were determined using a mass spectrometer Finnigan MAT TSQ 70.

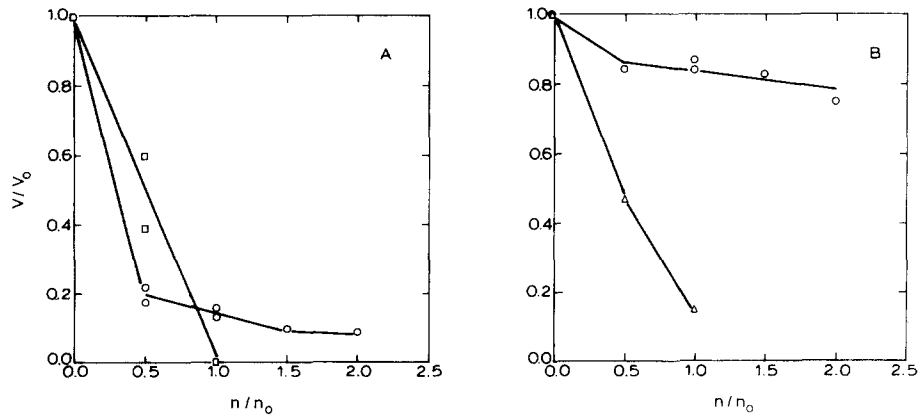


Fig. 1. Antimycin A sensitivity of the respiration as a function of the NADH oxidation rate. Mitochondria isolated from cells grown on glycerol in the absence of antimycin A were purified on a non-linear sucrose gradient and suspended at 0.5 mg ml^{-1} in the respiration buffer added with the NADH-regenerating system. (A) ○, respiration rate = 200 nat. O min^{-1} mg^{-1}. □, Respiration in the presence of 2 mM amytal = 170 nat. O min^{-1} mg^{-1}. (B) ○, Respiration rate = 25 nat. O min^{-1} mg^{-1}. △, Respiration in the presence of 2 mM SHAM = 19 nat. O min^{-1} mg^{-1}.

Results

Oxidation of external NADH

As previously shown, *C. parapsilosis* is able to grow on a non-fermentable carbon source in the presence of antimycin A, due to its capacity to oxidize cytoplasmic substrates such as NADH, NADPH and glycerol 3-P through the second respiratory chain described above. In mitochondria from organisms possessing an alternative pathway, the cytochrome chain appears to operate at its maximum rate, whereas the cyanide-insensitive pathway only operates when the input of reducing equivalents exceeds the capacity of the cytochrome pathway [12]. In this case, the total respiratory rate of the mitochondria is inferior to the sum of the respiratory capacity of each of the two chains. As already published, and opposite to this behaviour, in *C. parapsilosis* both pathways are additive [5]. Moreover, the proportion of antimycin-insensitive pathway varied as a function of the growth phase and the culture conditions: when cells were grown under normal conditions, i.e., in the absence of antimycin A, the involvement of the second chain varied from 10 to 25% of the total respiratory capacity in the early or in the late exponential phase respectively, but rose to 70 to 85%, respectively, when cells were grown in the presence of antimycin A.

In a first set of experiments we inquired about the partitioning of electron flux between both respiratory pathways when mitochondria oxidized external NADH. We titrated the antimycin A sensitivity of the respiration as a function of the oxidation rate in mitochondria isolated from *C. parapsilosis* grown under normal conditions. The respiration rate was modulated by limitation of substrate: NADH generation was adjusted by adding different amounts of glucose-6-phosphate dehydrogenase. The amount of antimycin A giving a complete inhibition of the main respiratory chain, referred to as n_o, was determined in the presence of 2 mM amytal which inhibits the alternative NADH dehydrogenase [4]. Fig. 1A and B show the antimycin A sensitivity of the NADH oxidation when the respiration rates were high and low respectively: at the maximal respiration rate ($VO_2 = 200$ nat. O min^{-1} mg^{-1}), the total inhibition of the main pathway was obtained at the n_o titer of antimycin A, and the remaining antimycin-insensitive oxidation represented 15% of the total respiratory capacity. In the second case ($VO_2 = 25$ nat. O min^{-1} mg^{-1}), the NADH oxidation rate represented 13% of the maximal rate, i.e., it did not exceed the capacity of the alternative pathway, and it can be seen that electron flow was not very sensitive to antimycin A, even at 2-fold n_o. This is in contrast to the results obtained when a single quinone pool operates [21,22]. However, prior addition of SHAM resulted in a total inhibition of respiration by antimycin A.

From these titrations it appeared that the alternative pathway of *C. parapsilosis* operated even when the oxidation rate was very low, suggesting that the partitioning of electron flux between both respiratory chains did not depend only on the reduction level of quinone pool, in contrast to the case in plant mitochondria [12].

In mitochondria from *C. parapsilosis* grown in the presence of antimycin A, it has been shown that the alternative pathway represented 80% of the total respiratory capacity of these mitochondria and that reducing equivalents were driven through this pathway until a cytochrome *c* which was the branch point between both respiratory chains [6,7]. The presence of a specific cytochrome *c* [7] involved in this electron transfer prompted us to inquire about cytochrome *b* reduction. In a previous paper, it was shown that although antimycin A-insensitive, the alternative pathway of *C. parapsilosis* was fully inhibited by high concentrations of myxothiazol or stigmatellin [5]. Since added together with antimycin A these inhibitors block reduction of cytochrome *b* [23], the basal reduction level was obtained by addition of antimycin A and stigmatellin before NADH (Fig. 2A, bar e), and maximal reduction level by addition of sodium dithionite (bar f). The steady-state levels of cytochrome *b* reduction resulting from NADH oxidation were measured under different conditions, comparing mitochondria isolated from cells grown in the absence (control mitochondria) or in the presence (antimycin-mitochondria) of antimycin A (Fig. 2A). From these measurements several lines of evidence can be drawn: (i) in control mitochondria, it can be seen that cytochrome *b* reduction level was related to the rate of NADH oxidation (compare bar a and bar b corresponding to low and high respiration rate respectively); as expected, antimycin A addition before NADH led to a higher cytochrome *b* reduction level (bar c), but addition of SHAM plus antimycin before NADH did not modify this reduction level (bar d). (ii) when the same experiments were performed with antimycin-mitochondria, addition of a low level of glucose-6-phosphate dehydrogenase promoted a higher cytochrome *b* reduction level than in control mitochondria (bar a), which however was enhanced by a further addition of NADH (bar b). Addition of antimycin A before NADH did not promote a higher cytochrome *b* reduction (bar c); however, addition of SHAM and antimycin A before NADH allowed to reduce more cytochrome *b* than in the presence of antimycin A alone (bar d). These results suggested that functioning of the alternative pathway involved the reduction of a specific *b*-type cytochrome which was fully reduced only in the presence of SHAM. To verify this hypothesis, mitochondria were incubated with antimycin A to allow full reduction of cytochromes *b* of the main respiratory chain; the mitochondrial suspension was then divided into two parts and one was

added with 2 mM SHAM. Then, cytochrome spectrum was recorded after NADH addition in both cuvettes; on mitochondria added with antimycin A + SHAM versus mitochondria incubated with only antimycin A (Fig. 2B). Under these conditions the spectrum shown looked quite typical of many respiratory *b*-type cytochromes, since it had absorbance maxima at 562 and 430 nm (β band was not correctly resolved).

Succinate oxidation

It was observed that, although succinate oxidation was fully sensitive to antimycin A when *C. parapsilosis* was grown in the absence of antimycin A (not shown), succinate can be oxidized through the second respiratory pathway at a very low rate by cells grown in the presence of antimycin A. This fact provided an opportunity to simultaneously measure oxygen uptake and steady-state reduction of the quinone pool by mitochondria from *C. parapsilosis* during succinate oxidation under state 4 conditions. Control mitochondria or antimycin-mitochondria were titrated with malonate to provide a progressive inhibition of succinate oxidation rate.

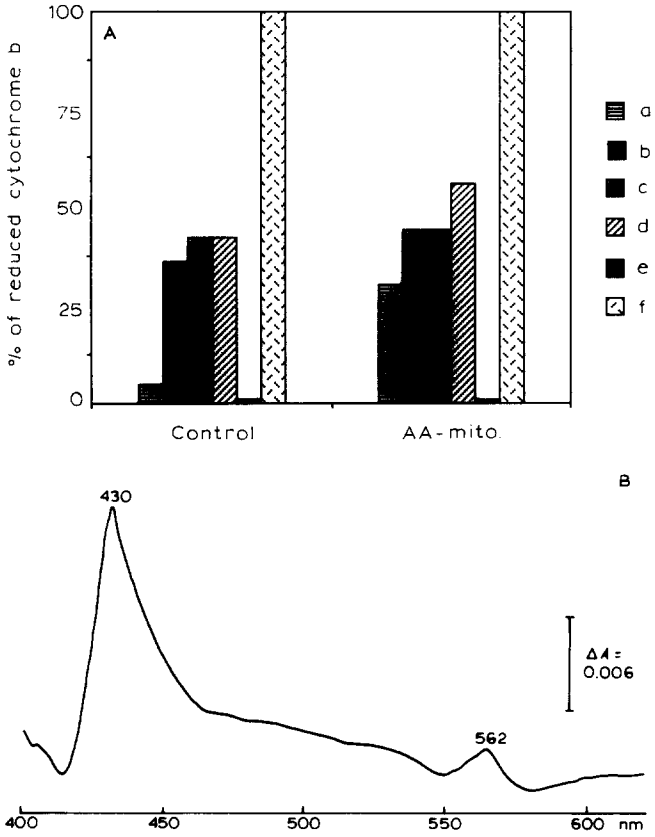


Fig. 2. Reduction levels of cytochrome *b*. Mitochondria were isolated from cells grown in the absence (control) or in the presence (AA-mito.) of antimycin A. They were purified on a sucrose gradient, suspended in the respiration buffer at 2 mg ml^{-1} and aerated. (A) Cytochrome spectra (reduced versus oxidized, steady-state) were recorded at 20°C under different conditions: (a) addition of the NADH-regenerating system and a low glucose-6-phosphate dehydrogenase concentration; (b) subsequent addition of 3 mM NADH; (c) addition of 5 μg antimycinA before 3 mM NADH; (d) addition of 2 mM SHAM+5 μg antimycin A before NADH; (e) addition of antimycin A+10 μg stigmatellin before NADH; (f)+sodium dithionite. (B) Cytochrome spectra was recorded on antimycin-mitochondria reduced with NADH after preincubation with 2 mM SHAM+5 mg antimycin A, versus antimycin-mitochondria reduced with NADH after preincubation with 5 μg of antimycin A.

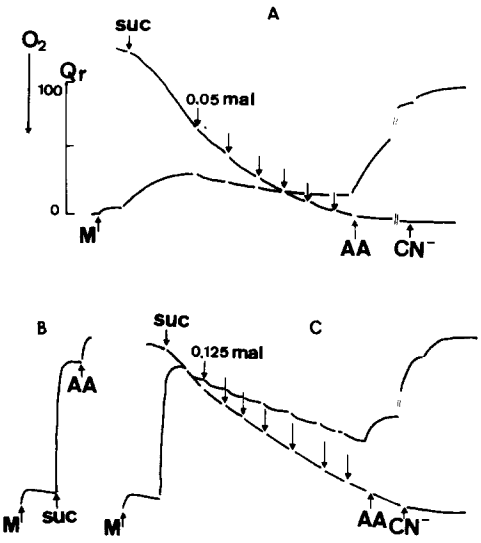


Fig. 3. Simultaneous titration of oxygen uptake and steady-state reduction level of quinone in mitochondria from *C. parapsilosis*. (A) Mitochondria were isolated from cells grown in the absence of antimycin A and suspended in the respiration buffer (0.45 mg ml^{-1}). Respiration (state 4) was initiated by addition of 5 mM succinate: actual value = 44 nat. O min^{-1} mg^{-1}. Malonate additions were done by increments of 0.05 mM. Further additions: 1 μM antimycin A, 10 mM KCN. (B, C) Mitochondria were isolated from cells grown in the presence of antimycin A and supended in the respiration buffer (1 mg ml^{-1}). Respiration was initiated by succinate. (B) Quinone reduction level following addition of 1 μg antimycin A. (C) Malonate additions were done by increments of 0.125 mM. Subsequent additions: 1 μM antimycin A, 10 mM KCN.

Fig. 3 shows the simultaneous measurement of oxygen uptake and the steady-state redox level of the quinone pool by *C. parapsilosis* mitochondria oxidizing succinate. Fig. 3A shows that the addition of succinate to control mitochondria resulted in a slow reduction of the quinone pool to only 25% of the level achieved upon anaerobiosis. Additions of low concentrations of malonate (up to 0.3 mM) led to progressive oxidation of the steady-state level of the quinone pool. The subsequent addition of antimycin A or myxothiazol resulted in a considerable reduction of quinone pool. When the experiment was repeated with antimycin-mitochondria, addition of succinate promoted an instantaneous reduction of 80% of the Q-pool. However, antimycin-mitochondria still retained some along the classical pathway, since antimycin A addition induced an additional reduction of the Q-pool (Fig. 3B). Malonate addition resulted in inhibition of respiration

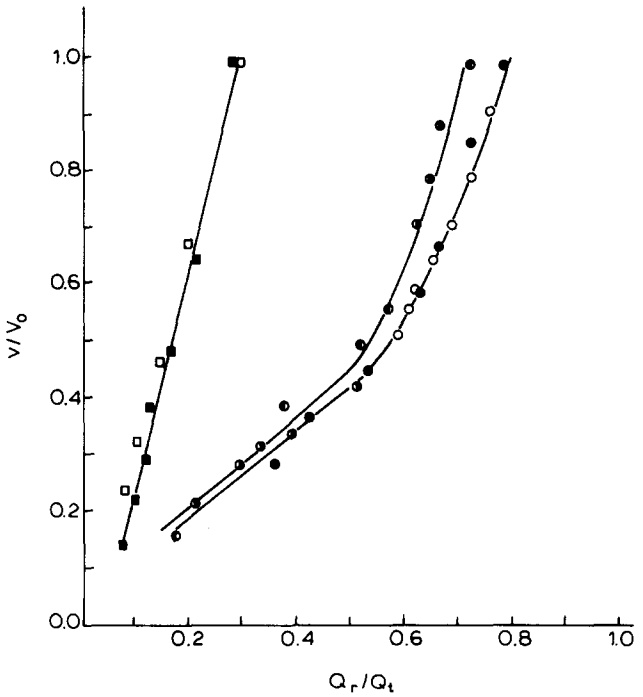


Fig. 4. Dependence of the respiratory rate on the quinone redox state in mitochondria from *C. parapsilosis*. Measurements were done as described in Fig. 3; plotting of two different experiments: □, ■, control mitochondria; ○, ●, antimycin-mitochondria; ◑, antimycin-mitochondria added with 2 μg antimycin A.

rate, while the quinone pool remained partly reduced. Further addition of antimycin A induced a partial reduction of the Q-pool with full reduction occurring only after the addition of 10 mM cyanide (Fig. 3C). It should be noted that, with antimycin-mitochondria, titration of succinate oxidation required 10-fold more malonate than with control mitochondria in order to achieve total inhibition of the respiratory rate. These different malonate sensitivities of succinate oxidation between both kinds of mitochondria were not due to a difference in succinate dehydrogenase activity or in malonate sensitivity of this enzyme. Indeed, experiments carried out in the presence of Triton X-100 showed that differences in titration profiles were only due to the dependence of the respiratory rate upon the succinate transport, since in the absence of permeability barrier, both kinds of mitochondria exhibited the same succinate dehydrogenase activity and the same malonate sensitivity (S. Zniber, unpublished results).

Fig. 4 illustrates the relationship between the oxidation rate (state 4) and the level of reduction of the quinone pool of mitochondria from cells grown in absence or in the presence of antimycin A. The data were plotted as the ratio V/V_0 (where V is the initial rate of oxygen uptake in the presence of malonate and V_0 the uninhibited rate) against the proportion of quinone reduced under steady-state conditions relative to total reducible quinone (Q_r/Q_t). In control mitochondria a linear relationship existed between these two parameters, a result comparable to that obtained with plant mitochondria lacking an alternative oxidase [15]. Such behaviour corresponds to the simple kinetic model of Kröger and Klingenberg [21,22], in which the rate of oxygen uptake is directly proportional to the redox poise of the quinone pool.

In antimycin-mitochondria the relationship between Q-pool reduction level and electron flux was non-linear, but somewhat different from that observed in plant mitochondria [15,16]. In plant mitochondria, the alternative pathway was not engaged to any significant extent until the level of reduction of the mitochondrial Q-pool reaches 30–40%. Below this level, rates of oxygen uptake via the alternative pathway were negligible. In mitochondria from *C. parapsilosis* grown in the presence of antimycin A, the curve showed that a low respiration rate (until 40–50% of the maximal rate) corresponded to the reduction of 50% of the total reducible quinone; higher oxidation rates leading to 80% reduction of the Q-pool. Addition of antimycin A to antimycin-mitochondria enhanced the phenomenon, and the behaviour of the mitochondrial response in this latter case suggested that the curve could be resolved in two distinct curves.

We have previously shown that in *C. parapsilosis* two different quinones occurred, ubiquinone 9 and another quinone species which is not a UQ_n [5]. This quinone was isolated from mitochondria purified on a sucrose gradient: its molecular weight is 514, as determined by mass spectrometry, with a global composition of $C_{33}H_{54}O_4$. Reduced and oxidized spectra are shown in Fig. 5: λ_{max} of the reduced forms were 279 nm and 289 nm for Q_x and UQ_9 respectively; λ_{max} of the oxidized forms were 274 nm and 276 nm for Q_x and UQ_9

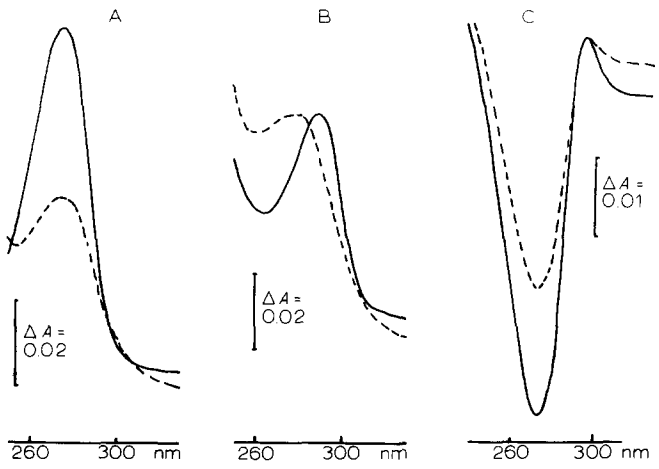


Fig. 5. Oxido-reduction spectra of Q_x and UQ_9. Quinones were suspended in ethanol (oxidized state, A), and then reduced with natrium borohydrure (B). (C) Reduced versus oxidized spectra. Dotted line, Q_x; continuous line, UQ_9.

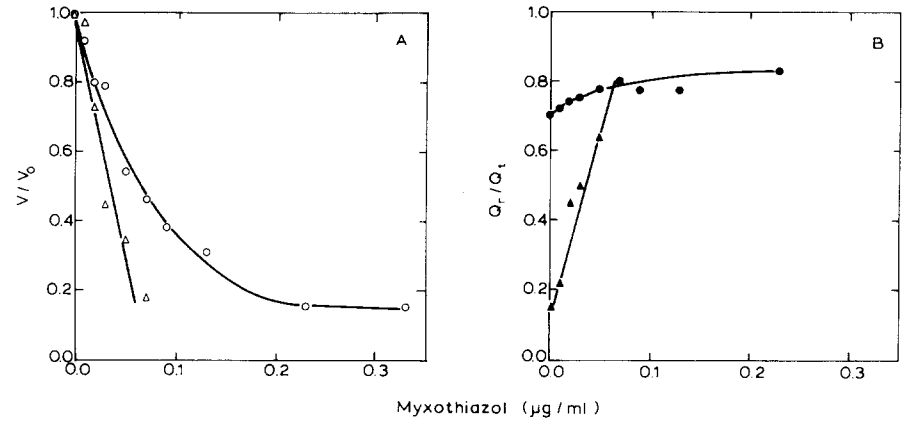


Fig. 6. Myxothiazol sensitivity of mitochondria as a function of the growth conditions. Mitochondria were suspended in the respiration buffer as described in Fig. 3. Myxothiazol additions were done during the course of the experiment. △ and ▲ represent ratios of V/V_0 and Q_r/Q_t respectively in control mitochondria. ○, ● represent ratios of V/V_0 and Q_r/Q_t respectively in antimycin-mitochondria.

respectively. Determination of the structure of this quinone is underway.

In previous experiments, in which NADH oxidation was titrated with myxothiazol, evidence was obtained for the presence of two classes of myxothiazol binding sites, one each being associated with the main and alternative respiratory chains, respectively [5]. A similar myxothiazol titration was carried out on antimycin-mitochondria oxidizing succinate and compared to control mitochondria (Fig. 6). It can be seen that, as shown previously with NADH [5], in control mitochondria succcinate oxidation was fully inhibited with 0.06 mg/ml of myxothiazol, whereas in antimycin-mitochondria inhibition of the second pathway required 10-fold more myxothiazol. In these latter mitochondria, although 70% of the quinone pool was reduced after succinate addition, the subsequent additions of myxothiazol resulted in a minor increase in reduction of this Q-pool, as is the case with the addition of antimycin A (Fig. 3).

Discussion

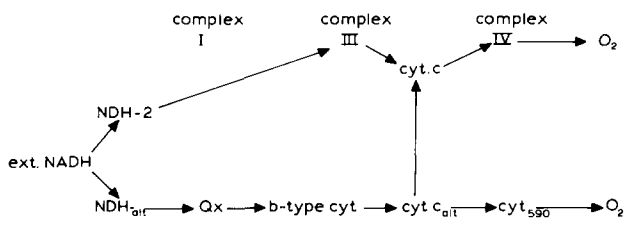
Data reported in this paper are concerned with the partitioning of electron flux between the main and the alternative respiratory chains in mitochondria of the yeast *C. parapsilosis*. The functional presence of this alternative chain was dependent on the culture conditions, i.e., when cells were harvested in stationary growth phase or when they were grown in the presence of antimycin A. Direct measurement of quinone reduction in mitochondria supporting only 10% of alternative pathway (control mitochondria) revealed that in this yeast, as in other systems, there is a linear relationship between the oxidation rate and the degree of reduction of the Q-pool, providing further confirmation of the proposal of Kröger and Klingenberg [21,22]. However, when a similar experiment was performed on antimycin-mitochondria (80% of alternative pathway), the shape of the curve was different. If, in plant mitochondria [15,16] or in *Rhodobacter capsulatus* [24], a considerable electron flux through alternative pathway is only observed when the Q-pool is significantly reduced, in antimycin-mitochondria from *C. parapsilosis* some electron flux is evident even at low Q_r values. Interestingly the profile could be resolved into two distinct curves, both of which showed linear dependencies on the two parameters.

The presence of an alternative pathway to molecular oxygen has been demonstrated in many organisms, all of which appear to branch off from the main respiratory pathway at the Q-pool level. In these systems, in which the quinone pool acts as an obligatory intermediate between dehydrogenases and oxygen, it has been observed that the total respiratory rate, with both pathways functioning simultaneously, is always inferior to the mathematical sum of the individual rates. From such observations, two models have been proposed. (i) According to the Bahr and Bonner model [25,26], the alternative pathway is only engaged upon full reduction of the Q-pool. (ii) According to the de Troostemberg and Nyns model [14], the partitioning of electron flux between the main and the alternative pathways is a function of their relative electron transport capacities. If this last partitioning has been proposed to be the case in the yeast *Saccharomycopsis lipolytica* [14], in plant mitochondondria the partitioning of electron flow between the cyanide-sensitive and cyanide-insensitive pathways is determined by the redox state of the ubiquinone pool, and the degree of engagement is dependent upon the degree of reduction of the Q-pool in a non-linear manner [16].

In *C. parapsilosis*, another type of electron flux

partitioning appears to occur. Several lines of evidence support this proposal. (i) The oxidation rate of a substrate (NADH or succinate) is equal to the sum of both individual respiration rates through the main and the alternative pathway, respectively. (ii) When the oxidation rate is lower than the capacity of the alternative pathway, electrons travelled preferentially through this route (Fig. 1). (iii) Addition of SHAM to antimycin A-treated mitochondria promoted an extra reduction of a *b*-type cytochrome (Fig. 2). (iv) In antimycin-mitochondria, the relationship between the respiration rate and the reduction reduction of quinone was non-linear and significant quinone reduction is observed even at low respiration rates. Thus in *C. parapsilosis*, partitioning of electron flux between both pathways seems different from the other two models. It is interesting to speculate that the two curves observed in plots of electron flow rate versus extent of quinone reduction obtained with antimycin A-treated cells (Fig. 4) could correspond to the functioning of a second quinone engaged in the alternative pathway. Some evidence in favour of this notion is given in the myxothiazol titrations shown in Fig. 6. It can be seen that addition of myxothiazol to antimycin-mitochondria resulted in a slight increase in the reduction level of quinone. A second quinone species ($C_{33}H_{54}O_4$) was shown to occur in these mitochondria. This second quinone, which should not be reducible in the presence of antimycin A but only in the presence of high concentration of myxothiazol or stigmatellin, should be constitutive of the second quinone pool and involved in the second respiratory chain of *C. parapsilosis*. Then electrons should be transferred to the cytochrome *c* specific of this chain, which appeared to have a lower mid-point redox potential than the cytochrome *c* of the main respiratory chain [7], with the possibility to be driven to this latter cytochrome *c* or to the alternative oxidase.

Thus, the alternative pathway of *C. parapsilosis* should be a complete respiratory chain, functioning in parallel to the main one, and we hypothesized that a third type of partitioning of electron flux could exist in this yeast, occurring upstream of complex III with the additional possibility of merging into the main respiratory chain at the complex IV level (Scheme I).



Scheme 1.

Acknowledgements

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References

1 Guérin, M., Camougrand, N., Caubet, R., Zniber, S., Velours, G., Manon, S., Guélin, E. and Cheyrou, A. (1989) Biochimie 71, 887–902.
2 Degn, H., Llyod, D. and Hill, G.C. (eds.) (1977) Function of Alternative Terminal Oxidases. Pergamon, Oxford.
3 Moore, A. and Siedow, J. (1991) Biochim. Biophys. Acta 1059, 121–140.
4 Camougrand, N., Cheyrou, A., Henry, M.F. and Guérin, M. (1988) J. Gen. Microbiol. 134, 3195–3204.
5 Camougrand, N., Zniber, S. and Guérin, M. (1991) Biochim. Biophys. Acta 1057, 124–130.
6 Guérin, M. and Camougrand, N. (1986) Eur. J. Biochem. 159, 519–524.
7 Camougrand, N., Velours, J., Denis, M. and Guérin, M. (1993) Biochim. Biophys. Acta 1143, 135–141.
8 Camougrand N., Velours, G. and Guérin, M. (1991) A. von Leeuwenhoek 59, 235–241.
9 Camougrand, N., Velours, G. and Guérin, M. (1986) Biol. Cell. 58, 71–78.
10 Yagi, T. (1991) J. Bioenerg. Biomembr. 23, 211–225
11 Yagi, T. (1993) Biochim. Biophys. Acta 1141, 1–17
12 Cottingham, I.R. and Moore, A.L. (1983) Biochim. Biophys. Acta 724, 191–200
13 Von Jagow, G., Weiss, H. and Klingenberg, M. (1973) Eur. J. Biochem. 33, 140–157
14 De Troostembergh, J.C. and Nyns, E.J. (1978) Eur. J. Biochem. 85, 423–432.
15 Moore, A.L., Dry, I.B. and Wiskich, J.T. (1988) FEBS Lett. 235, 76–80.
16 Dry, I.B., Moore, A.L., Day, D.A. and Wiskich, J.T. (1889) Arch. Biochem. Biophys. 273, 148–157.
17 Moore, A.L., Day, D.A., Dry, I.B. and Wiskich, J.T. (1990) in Highlights in Ubiquinone Research (Lenaz, G., et al., eds.), pp. 170–174, Taylor & Francis, London.
18 Camougrand, N., Mila, B., Velours, G., Lazowska, J. and Guérin, M. (1988) Curr. Genet. 13, 445–449.
19 Guérin, B., Labbe, P. and Somlo, M. (1979) Methods Enzymol. 55, 149–159.
20 Singer, T.P., Oestreicher, G., Hogue, P., Contrairas, J. and Brandao, I. (1973) Plant Physiol. 52, 616–621.
21 Kröger, A. and Klingenberg, M. (1973) Eur. J. Biochem. 34, 358–368.
22 Kröger, A. and Klingenberg, M. (1973) Eur. J. Biochem. 39, 313–323.
23 Von Jagow, G., Ljungdahl, P.O., Graf, P., Ohnishi, T., and Trumpower, B.L. (1984) J. Biol. Chem., 259, 6318–6326.
24 Zannoni, D. and Moore, A.L. (1990) FEBS Lett. 271, 123–127.
25 Bahr, J.T. and Bonner, W.D. (1973) J. Biol. Chem. 248, 3441–3445.
26 Bahr, J.T. and Bonner, W.D. (1973) J. Biol. Chem. 248, 3446–3450.