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Electron competition process in respiratory chain: Regulatory mechanisms and physiological functions

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ABSTRACT

In mitochondria isolated from the yeast *Saccharomyces cerevisiae*, under non-phosphorylating conditions, we have previously shown that there is a right of way for electrons coming from the external NADH dehydrogenase, Nde1p. In this work, we show that the electron competition process is identical under more physiological conditions i.e. oxidative phosphorylation. Such a competition generates a priority for cytosolic NADH reoxidation. Furthermore, this electron competition process is associated with an energy wastage (the "active leak") that allows an increase in redox equivalent oxidation when the redox pressure increases. When this redox pressure is decreased, i.e. under phosphorylating conditions, most of this energy wastage is alleviated. By studying mutant strains affected either in respiratory chain supramolecular organization or in electron competition activity, we show that the respiratory chain supramolecular organization is not responsible for the electron competition processes. Moreover, we show two distinct relationships between the respiratory rate and the quinone redox state that seem to indicate two quinone pools that are involved in the electron right of way. Indeed, the more reduced pool would be associated to the electron right of way for the external dehydrogenases whereas the less reduced pool would be associated to the electron right of way for the internal dehydrogenases.

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1. Introduction

The yeast *Saccharomyces cerevisiae* lacks transhydrogenase activity [1], and the redox couples NAD^+/NADH and $NADP^+$/NADPH cannot pass through the inner mitochondrial membrane. Hence, to maintain the redox balances, the reduced coenzymes must be reoxidized in the compartment in which they are produced. In contrast to NADPH turnover, which occurs essentially in the cytosol [1], systems for NADH turnover in the cytosol as well as in mitochondria are required both during aerobic and anaerobic growth conditions. Indeed, several processes result in production of NADH, i.e. several processes are, contrary to ethanol fermentation, not redox neutral. The synthesis of one mole of glycerol, the second major by-product of *S. cerevisiae* cells fermenting glucose, results in the consumption of one mole of NADH, whereas other by-products such as acetate lead to the production of cytosolic NADH. The largest part of excess cytosolic NADH formation is connected to biomass production [2]. Synthesis of proteins, nucleic acids and even the highly reduced lipids are associated with assimilatory NADH production. In particular, NADH is generated in the biosynthetic pathways of amino acid synthesis [2]. Anaerobically, the only mean by which *S. cerevisiae* can reoxidize surplus production of NADH is by glycerol production [3]. Aerobically, several systems exist for conveying excess cytosolic NADH to the mitochondrial electron transport chain in *S. cerevisiae*. The two most important systems in this respect seem to be the external NADH dehydrogenase (Nde1p/Nde2p) [4,5] and the glycerol 3-phosphate shuttle [6,7]. The Nde1p/Nde2p system, which is localized in the inner mitochondrial membrane with the catalytic sites projecting toward the intermembrane space, has been shown to directly oxidize cytosolic NADH [4,5]. The glycerol 3-phosphate shuttle system, which involves the FAD dependent Gut2p [8], is situated in the mitochondrial inner membrane with the catalytic site projecting toward the intermembrane space and has been shown to be active in maintaining a cytosolic redox balance [6]. In this system, the co-factor NADH is oxidized to NAD^+ by the cytosolic glycerol-3-phosphate dehydrogenase (Gpd1p) when catalyzing the reduction of dihydroxyacetone phosphate (DHAP) to glycerol 3-phosphate. Thus, glycerol 3-phosphate delivers its electrons to the respiratory chain via Gut2p with the subsequent regeneration of DHAP.

We have shown that when NADH is produced by cytosolic NAD^+-dependent dehydrogenases (such as the glyceraldehyde-3-phosphate dehydrogenase or the cytosolic alcohol dehydrogenase), it is channeled from these dehydrogenases to the inner mitochondrial membrane through porin [9–11]. This channeling mechanism would directly

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deliver NADH produced in the cytosol to NADH dehydrogenases located on the external side of the inner mitochondrial membrane. Moreover, using mitochondria isolated from a wild type strain and different mutant strains in which deletion of the genes leads to the loss of one of the external dehydrogenase activities we evidenced kinetic interactions between Nde1p/Nde2p and Gut2p. Thus, the deletion of either one of the external dehydrogenases caused an increase in the efficiency of the remaining enzyme [12]. In addition, we have shown that the activation of NADH dehydrogenase inhibits the Gut2p in such a manner that, at a saturating concentration of NADH, glycerol 3-phosphate is not used as a respiratory substrate. This effect is not a consequence of a direct action of NADH on Gut2p activity because both NADH dehydrogenase and its substrate are needed for Gut2p inhibition [12]. Complementary studies on the functionally isolated enzymes demonstrated that neither Nde1p nor Nde2p directly inhibits Gut2p. Thus, the inhibition of glycerol 3-phosphate oxidation may be caused by competition for the entrance of electrons into the respiratory chain [13]. Using single deletion mutants of Nde1p or Nde2p, we showed that glycerol 3-phosphate oxidation *via* Gut2p is fully inhibited when NADH is oxidized via Nde1p, whereas only 50% of glycerol 3-phosphate oxidation is inhibited when Nde2p is functioning. By comparing respiratory rates with different respiratory substrates, we show that electrons from Nde1p are favored over electrons coming from Ndip (internal NADH dehydrogenase) and that when electrons come from either Nde1p or Nde2p and succinate dehydrogenase, their transfer through the respiratory chain is shared to a comparable extent [13]. This suggests a very specific competition for electron entrance into the respiratory chain.

The above-mentioned study raised two distinct questions, which we address in this report: (i) is the kind of electron supply competition due to a particular supramolecular organization of the respiratory chain ? (ii) are there specific functional consequences of such processes in terms of regulation, oxidative phosphorylation efficiency or reactive oxygen species production?

2. Materials and methods

2.1. Yeast strains and growth conditions

The *S. cerevisiae* strains used in this study were: W303-1A (*ade*2-1, *his*3-11, *leu*2-3, 112*trp*1-1a, *ura*3-1, *can*100); Δnde1 (ade2-1, his3-11, 112trp1-1a, can100, Δnde1 ::TRP1) and Δcrd1 (ade2, his3, leu2, trp1, ura3, cls1 ::KANMX4). Cells were grown aerobically at 28 °C as chemostat cultures, in the following medium: yeast extract 1% (w/v), bactopeptone 1% (w/v), KH_2PO_4 0.1% (w/v), $(NH_4)_2SO_4$ 0.12% (w/v), adenine 0.01% (w/v) and glucose 0.5% (w/v) as the carbon source; pH5.5. The cells were harvested after glucose depletion i.e. during respiratory growth on ethanol.

2.2. Mitochondria preparation

Yeast mitochondria were prepared by the enzymatic method as described previously [14] and suspended in the mitochondrial buffer: 0.65 M mannitol, 0.36 mM EGTA, 10 mM Tris–maleate, 5 mM Tris–Phosphate, pH 6.8.

2.3. Respiration assay

Oxygen consumption was measured at 28 °C in a 2 ml thermostatically controlled chamber equipped with a Clark oxygen electrode (Gilson) connected to a recording device that provides a display of the oxygen consumption rate. Mitochondria (0.5 mg ml^{-1}) were incubated in the mitochondrial buffer (described above). Unless specified, substrates were used at the following concentrations: NADH (10 mM), glycerol-3-phosphate (10 mM), ethanol (100 mM) and succinate (10 mM).

2.4. Measurement of glycerol-3-phosphate consumption flux (JDHAP) and of NADH consumption flux (JNAD$^+$)

DHAP was measured fluorimetrically in 7% $HClO_4$/25 mMEDTA extracts neutralized with KOH 2 M, MOPS 0.3 M as described previously in Bergmeyer [15]. NAD^+ was measured fluorometrically in neutralized 7% $HClO_4$/25 mM EDTA extracts as described previously [15].

2.5. Enzymatic activities

NADH-ferricyanide reductase and glycerol-3-phosphate-ferricyanide reductase activities were assayed in mitochondria in the presence of various concentrations of glycerol-3-phosphate with or without 10 mM NADH. The reaction was started by the addition of 1 mM ferricyanide (Fe(CN)6). Ferricyanide reduction was followed at 420 nm ($\varepsilon = 0.86$ mM^{-1} cm^{-1}).

2.6. Measurement of Δp by labeled probes distribution

Matrix space was determined by using [^{3}H]water and inner membrane impermeable [^{14}C]mannitol. ΔΨ and ΔpH were determined by the distribution of [^{3}H]TPMP+ and [^{3}H]Acetate, respectively. [^{3}H]TPMP$^+$ is a lipophilic cation and its binding coefficient was determined in our laboratory as being equal to 0.38 [16]. Routinely, after equilibration (3 min), mitochondria were separated from the medium by rapid centrifugation (30 s), through a silicon oil layer and then treated as described previously [17].

2.7. Measurement of ATP synthesis flux (JATP)

Isolated mitochondria (0.5 mg ml^{-1}) were suspended in the mitochondrial buffer (see above). After addition of the respiratory substrates and 1 mM ADP, both oxygen consumption and ATP synthesis rates were measured. Samples were removed every 20 s and precipitated in 7% HCLO4/25 mM EDTA and then neutralized with KOH 2 M, MOPS 0.3 M. The ATP content in these samples was determined with the ATPlite 1step from PerkinElmer®. In a parallel experiment, oligomycin (1.5 µg mg^{-1} protein) was added to the mitochondrial suspension to determine the non-oxidative ATP synthesis rate.

2.8. Measurement of reactive oxygen species

The rate of H_2O_2 production was determined by monitoring the oxidation of the fluorogenic indicator amplex red in the presence of horseradish peroxidase. The concentrations of horseradish peroxidase and amplex red in the incubation medium were 5 U/ml and 1 µM, respectively. Fluorescence was recorded at the following wavelengths: excitation 560 nm and emission 584 nm. A standard curve was obtained by adding known amounts of H_2O_2 to the assay medium in the presence of the reactants. Mitochondria (0.3 mg protein ml^{-1}) were incubated in the respiratory medium, at 28 °C and H_2O_2 production was initiated by substrate addition. H_2O_2 production rate was determined from the slope of a plot of the fluorogenic indicator versus time [18].

2.9. BN-PAGE experiments

BN-PAGE experiments were carried out as described by Schägger [19]. Mitochondria (1 mg of protein) were incubated for 45 min at 4 °C with 0.1 mL of digitonin at a digitonin/protein ratio of 8 g/g. The extracts were centrifuged at 4 °C for 15 min at 20 000 g, and aliquots of the supernatant (50 µL) were immediately loaded on the top of a 3–13% polyacrylamide gel [19]. After electrophoresis, the gel was divided into strips. Then these strips were incubated in different

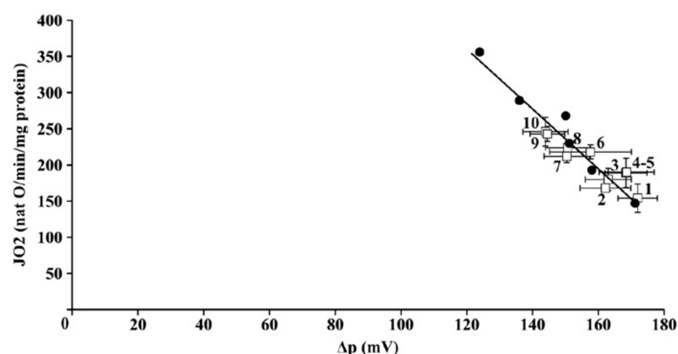


Fig. 1. A unique relationship between non-phosphorylating respiratory rate and the protonmotive force when the respiratory rate was modulated with either multiple substrates or a protonophore. Mitochondria (3 mg ml−1) were incubated in mitochondrial buffer as described in "Materials and methods". Respiratory rates were modulated using various respiratory substrates. Ethanol (1), glycerol-3-phosphate (2), NADH + ethanol (3), NADH (4) succinate (5) NADH + succinate (6) succinate + ethanol (7) NADH + glycerol-3-phosphate (8) ethanol + glycerol-3-phosphate (9) succinate + glycerol-3-phosphate (10). Here are also represented conditions with ethanol uncoupled by increasing amounts of CCCP (100 to 360 nM) (●). Matricial volume, ΔpH and ΔΨ were measured under the same conditions in parallel experiments as described in Materials and methods. Results are means ± S.D. of at least three independent experiments carried out on three different mitochondrial preparations.

solutions at room temperature during 20 min to 1 h to reveal in gel activity.

In order to reveal in gel ATPase activity, gel strips were incubated in a solution of 5 mM ATP, 5 mM $MgCl_2$, 0.05% lead acetate, 50 mM glycine–NaOH pH 8.4 to reveal the ATPase activity. For the cytochrome-*c*-oxidase activity strips were incubated in the next solution (diaminobenzidin 0.6‰ (w/v), bovin heart cytochrome *c* 1.2‰ (w/v), catalase 1nM, H_2PO_4 50 mM, pH:7). For dehydrogenase activities strip were incubated with dehydrogenase substrates (NADH 400 µM (for Ndip and Nde1/2p), succinate 100 mM, glycerol-3-phosphate 50 mM) with iodonitroblue tetrazolium formazan 0.5 mg ml^{-1}) in phosphate buffer 50 mM pH: 7.4 [19].

2.10. Quinones redox state

Frozen mitochondria were used to measure oxidized and reduced coenzyme Q6 contents. After dissolving and extraction in 2-propanol, CoQ6 was detected by reverse-phase high performance liquid chromatography with electrochemical detection as described in [20].

3. Results and discussion

3.1. From the electron competition process to the active leak

Most studies conducted on isolated mitochondria are done in the presence of a sole substrate. However, *in situ*, mitochondria are in the presence of multiple substrates and we have previously investigated mitochondrial substrate oxidation rates in the presence of various substrates in non-phosphorylating conditions (state 4). We have shown that in mitochondria isolated from wild type yeast, electrons coming from the external NADH dehydrogenase have the right of way on electrons coming from either the internal NADH dehydrogenase or the glycerol-3-phosphate dehydrogenase. In contrast, electrons coming from succinate are shared together with the ones coming from external NADH dehydrogenase [13]. Moreover, we observed that electrons provided by internal dehydrogenases (NADH or succinate dehydrogenase) have the right of way on electrons coming from glycerol-3-phosphate dehydrogenase [21]. While investigating the respiratory chain electron competition process, we observed that the non-phosphorylating respiratory flux could increase by 40% depending on the respiratory substrate(s). This is an unusual observation, since in *S. cerevisiae*, all the dehydrogenases considered give their electrons to the quinone pool and consequently the number of coupling sites is constant. Under these conditions, any change in the respiratory rate would reflect a change in the inner membrane proton permeability (proton leak) [22–28] or a decrease in the proton pumps efficiency (redox slipping) [29–31]. We have shown that the increase in respiratory rate is associated with a decrease in protonmotive force (Fig. 1). The relationship between these two parameters is similar to the one obtained with a protonophore. Furthermore, the decrease in proton motive force is strictly proportional to the activity of the membranal dehydrogenases and independent of the proton pumps. In conclusion, we have shown that the membranal dehydrogenases can generate what we propose to call an "active proton leak" [21]. This is a very original mechanism where enzymes that are not involved in energy-dependent proton movements across the inner membrane are able to generate a proton leak. Such a process might be of importance in physiological situations where the mitochondrial substrates redox state increases, leading to a more reduced quinone pool and thus an increase in ROS production that is well known to be deleterious to the cell. We thus investigated a possible relationship between ROS production rate, quinone redox state and respiratory rate under these experimental conditions. Table 1 shows that the quinone redox state is maintained constant in the presence of two respiratory substrates such as G3P/EtOH or G3P/succinate in comparison with their redox state with G3P alone. Further, the ROS production rate is also almost constant but if one considers the most important parameter, which is the amount of ROS produced per oxygen consumed, owing to the active leak process, there is a decrease in the peroxidic yield (i.e. JH_2O_2/JO_2).

3.2. What about electrons competition in more physiological conditions i.e. state 3

All these experiments were done in non-phosphorylating conditions and we asked whether such a competition was observed in more physiological conditions i.e. phosphorylating (state 3). We assessed both respiratory flux (JO_2) and, when doable, substrate consumption fluxes. Two fluxes were easily estimated: NADH consumption ($JNAD^+$) and glycerol 3-phosphate oxidation (J_{DHAP}). By comparing these fluxes in each experimental condition (Table 2), we were able to show that, in state 3, the competition for electron supply to the respiratory chain did exist and was identical to the one described in state 4 i.e. external NADH dehydrogenase electrons have the right of way on electrons coming from either the internal NADH dehydrogenase or the glycerol-3-phosphate dehydrogenase while electrons coming from succinate are shared together with the ones coming from external NADH dehydrogenase. Moreover, electrons provided by internal dehydrogenases have

Table 1
Respiratory rates, quinone redox state and ROS production under non-phosphorylating conditions. Mitochondria (3 mg ml^{-1}) were incubated in mitochondrial buffer as described in "Materials and methods". Respiratory rates were modulated using various respiratory substrates. Results are means ± S.D. of at least three independent experiments carried out on three different mitochondrial preparations.

Substrate	JO_2 (natO min^{-1} mg^{-1}prot)	Quinones (reduced/total (%))	ROS (pmol min^{-1} mg^{-1})	JH_2O_2/JO_2 (pmolH_2O_2 $natO^{-1}$)
G3P	168 ± 5	25 ± 5	686 ± 71	4.1
G3P/EtOH	243 ± 11	29 ± 8	615 ± 149	2.5
G3P/Succinate	246 ± 20	22 ± 4	616 ± 46	2.5

Table 2
State 3 respiratory rate, dihydroxyacetone phosphate and NAD^+ production flux in mitochondria isolated from wild type yeast. Mitochondria (3 mg ml^{-1}) were incubated in mitochondrial buffer as described in "Materials and methods". Respiratory rates were modulated using various respiratory substrates. Results are means ± S.D. of at least three independent experiments carried out on three different mitochondrial preparations.

Substrates	JO_2 (natO min^{-1} mg^{-1}prot)	$JNAD^+$ (nmol NAD^+ min^{-1} mg^{-1}prot)	J_{DHAP} (nmol DHAP min^{-1} mg^{-1}prot)
NADH	487 ± 22	474 ± 27	–
G3P	277 ± 12	–	277 ± 17
EtOH	349 ± 28	–	–
Succinate	185 ± 13	–	–
NADH/G3P	655 ± 32	630 ± 28	12 ± 5
NADH/EtOH	464 ± 22	462 ± 26	–
NADH/Succinate	559 ± 38	371 ± 27	–
G3P/EtOH	580 ± 28	–	91 ± 12
G3P/Succinate	460 ± 22	–	232 ± 17

the right of way on electrons coming from glycerol-3-phosphate dehydrogenase. This shows that the electrons competition process does have a physiological meaning.

Furthermore, Table 2 shows that, comparable to what was seen in state 4, the respiratory flux varies when different or multiple substrates are used. Since in state 4 we had shown that this increase in respiratory rate was associated to an active leak process i.e. an increase in the proton permeability of the inner mitochondrial membrane, we raised the question of a possible leak process in state 3. The best indicator of the proton leak of the inner mitochondrial membrane, in phosphorylating conditions, is the oxidative phosphorylation yield (i.e. the ratio between ATP synthesis flux and oxygen consumption flux). We thus assessed the relationship between J_{ATP} and JO_2 under our experimental conditions of multiple substrates (NADH with increasing concentrations of glycerol-3-phosphate) and with a slight amount of uncoupler (NADH). Fig. 2 shows that (i) when JO_2 increases there is an associated increase in J_{ATP} even though the oxidative phosphorylation yield slightly decreases; (ii) mild uncoupling with NADH as substrate did not increase the respiratory rate but led to an important decrease in J_{ATP} and thus in the oxidative phosphorylation yield. In conclusion, under phosphorylating conditions, even if the active leak is present an increase in J_{ATP} is associated to the JO_2 increase, leading to very little decrease in the oxidative phosphorylation yield.

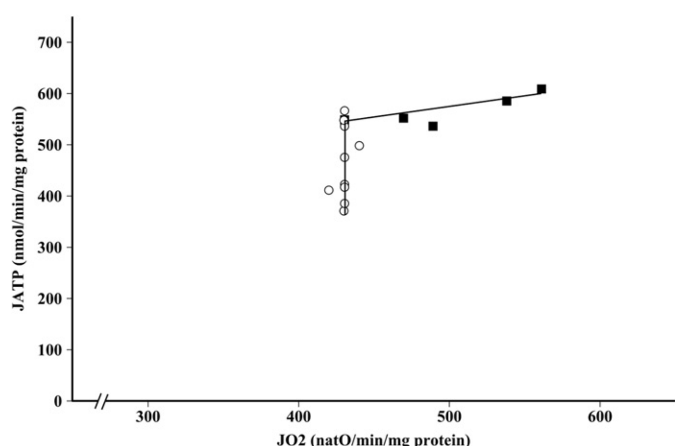


Fig. 2. Two distinct relationships between ATP synthesis and oxygen consumption when there are modulated with either multiple substrates or a protonophore. Mitochondria (0.5 mg ml^{-1}) were incubated in mitochondrial buffer (see "Materials and methods"). The respiratory substrates were added in the presence of ADP (1 mM) and ATP synthesis and oxygen consumption were assessed with increasing concentrations of glycerol-3-phosphate (5 to 20 mM) in the presence of NADH (10 mM) (■). ATP synthesis and oxygen consumption were also assessed with increasing concentrations of CCCP (50 to 250nM) in the presence of NADH (10 mM) (○). Results are means ± S.D. of at least three independent experiments carried out on three different mitochondrial preparations.

3.3. Is the respiratory chain supramolecular organization responsible for the electrons competition process?

Historically, the respiratory chain has been defined as an ensemble of complexes (I, II, III, and IV), some of which (I, III and IV) couple redox reaction to proton extrusion. Such complexes are called coupling sites. It has recently been shown that mitochondrial complexes are associated into supramolecular ensembles in a whole range of organisms [32–34]. General functional advantages of supercomplexes compared to separated complexes are catalytic enhancement, substrate channeling, sequestration of reactive intermediates, and structural stabilization of one specific complex by supercomplex formation. Quite a few studies have been realized on mitochondria isolated from the yeast *S. cerevisiae*, both at the structural and functional level. Structural studies have shown that the formation of yeast respiratory chain supercomplexes (i.e. association of complexes III and IV) depends on the growth conditions [32]. As stated above, *in situ*, mitochondria are subjected to multiple substrates and functional studies from our laboratory have shown that electrons coming from certain dehydrogenases have the right of way on electrons coming from other dehydrogenases [12,13]. These results are in close agreement with structural studies showing a supramolecular organization of the respiratory chain and led us to hypothesize that these supercomplexes can interact specifically with either complex II to form a respiratory chain using succinate or with another supercomplex containing most of the dehydrogenases. Such a supramolecular organization of the respiratory chain would be in close agreement with functional studies.

The interactions between the bc1 complex and cytochrome oxidase, opposite to what is seen for the ATP synthase [35] do not seem to be due to protein–protein interactions. The non-essential subunits of complex III and IV are not involved in the formation of these complexes [36,37]. The only case where a destabilization of these complexes has been shown is in a yeast mutant deleted for the cardiolipid synthase, indicating that cardiolipids are necessary for the supramolecular organization of the respiratory chain [37]. In order to determine whether the electron competition is in any way due to the supramolecular organization of the respiratory chain, we used mitochondria isolated from a strain in which the electron competition process was impaired (Δnde1) or mitochondria isolated from a strain in which the supramolecular organization of the respiratory chain was impaired (Δcrd1).

First we assessed the electron competition in the Δcrd1 and Δnde1 mitochondria compared to the wild type mitochondria (Fig. 3). Respiratory rate and either J_{NAD+} or J_{DHAP} were assessed for multiple substrates combinations.

3.3.1. WT versus Δnde1

The respiratory fluxes assessed for any substrates or substrates combinations are similar for the wild type and the mutant mitochondria. In the Δnde1 mitochondria, the electron competition process is quite different from what is seen in the wild type mitochondria. The NADH oxidation flux by nde2p is shared when this substrate is associated with either ethanol or glycerol 3 phosphate. In the presence of succinate, the NADH oxidation flux seems to be less that the succinate oxidation flux. Indeed, in the Δnde1 mitochondria, for the substrate combinations NADH/G3P, NADH/ethanol and G3P/succinate each respiratory substrate participates for half the respiratory flux. For the substrate combinations NADH/succinate and G3P/ethanol, the internal dehydrogenases seem to have the right of way on the electron transfer (there is an important decrease in NADH and G3P oxidation fluxes).

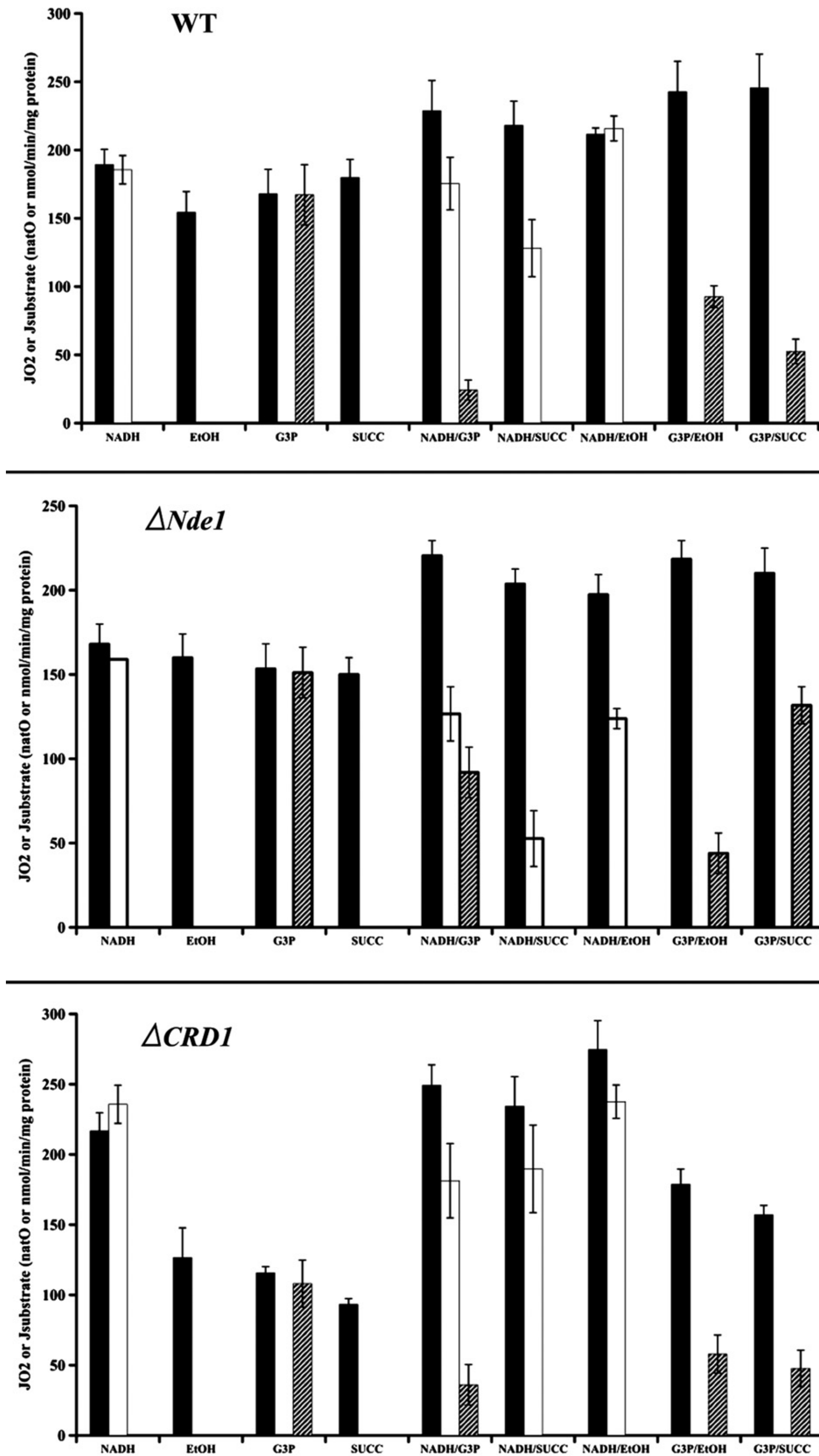


Fig. 3. Respiratory rate, NAD^+ and DHAP production flux. Isolated mitochondria were incubated (0.5 mg ml^{-1}) in the mitochondrial buffer (see Materials and methods). Oxygen consumption (natO min^{-1} mg^{-1} protein) (■) was measured at 28 °C with a Clark electrode. Acidic extraction of aliquot were analyzed enzymatically in order to quantifying fluorimetrically the NAD^+ (□) and DHAP production rate (▨) (nmol min^{-1} mg^{-1}). Results are means ± SD of at least three independent experiments.

3.3.2. WT versus Δcrd1

Even though there is a slight decrease in the respiratory fluxes with either G3P, EtOH or succinate in the Δcrd1 mitochondria, the electron competition process assessed in this strain is clearly similar to what is assessed in the wild type strain. Indeed, in both strains, electrons coming from NADH have the right of way on electrons coming from G3P or EtOH.

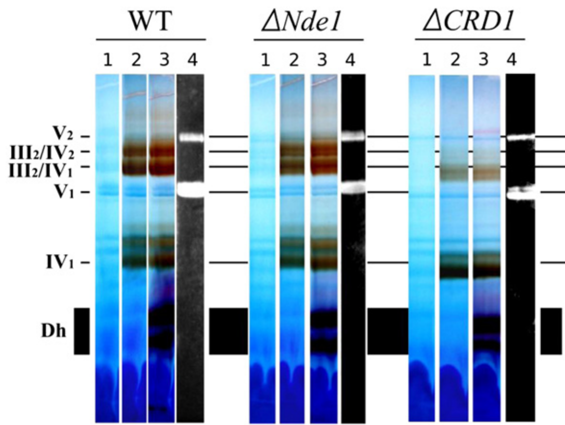


Fig. 4. Characterization of the respiratory chain supramolecular organization by BN-PAGE and in gel activities. Isolated mitochondria were treated with Digitonin (8 g/g) during 45 min. After migration in polyacrylamide gradient gel (3%–13%). Enzymatic activities of native protein were used with colored reagents to localize the supercomplex organization of the oxidative phosphorylation system. According to this technique ATP synthase (V_2 and V_1), supercomplexes (IIIx/IVx), cytochrome-*c*-oxidase (IV_1) and dehydrogenases (Dh) were localized. Results are representative of three independent experiments.

In order to determine a possible involvement of the respiratory chain supramolecular organization in the electron competition process, we next examined the supramolecular organization in the Δcrd1 and Δnde1 isolated mitochondria. BN-page analysis (Fig. 4) showed that the migration pattern of the supercomplexes in the wild type and Δnde1 mitochondria are pretty similar. Indeed, there is no difference between the III2/IVx and the dehydrogenase supercomplexes. Moreover, in the Δcrd1 mitochondria, as previously shown, there are very little respiratory chain supramolecular complexes. The results obtained here support the fact that there is probably no relationship between the electron competition process and the respiratory chain supramolecular organization.

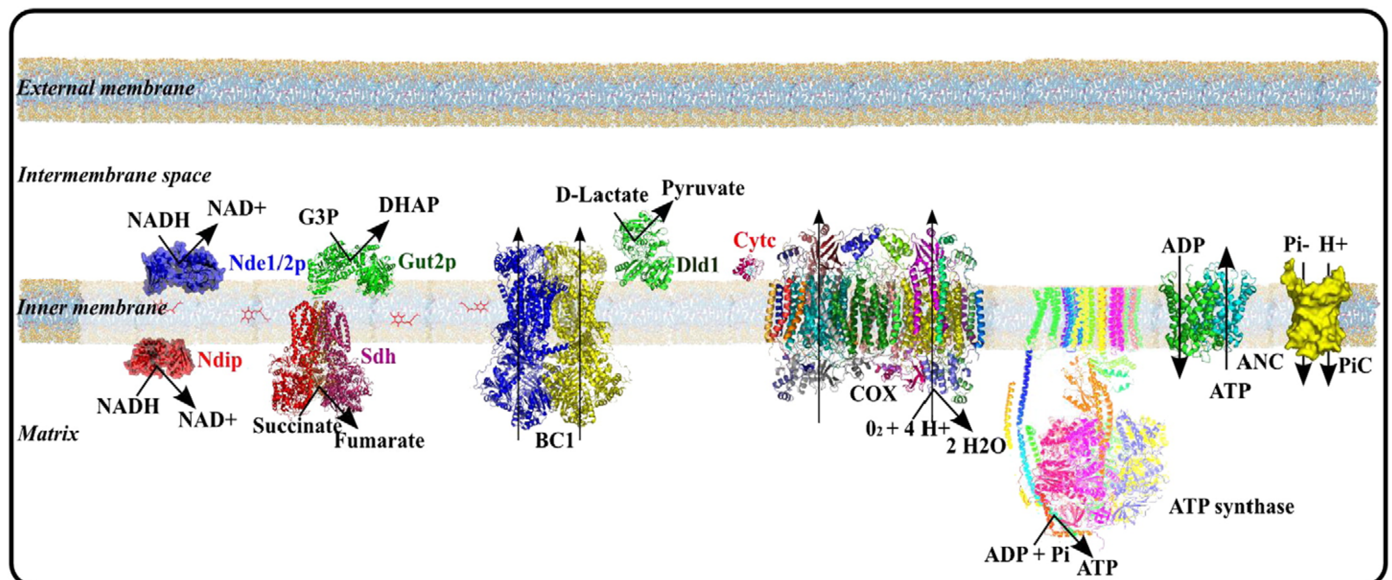
3.4. Does the electron competition process originate at the level of the quinone redox state?

Since the electron competition process did not seem to be due to the supramolecular organization of the respiratory chain and since all the dehydrogenases that we have studied give their electrons to the quinone pool (Scheme 1), we hypothesized that the quinone pool redox state could be involved in the regulation of the electron competition process. The quinone redox state was thus assessed in non-phosphorylating conditions with the different substrates and substrate combinations. Fig. 5 shows that the quinone redox state varies greatly depending on the respiratory substrate and substrate combinations. Indeed, with NADH as substrate and any substrate combination with NADH, the quinones are very much reduced whereas with glycerol-3-phosphate, only 23% of the quinones are reduced. Moreover, with the substrates of the internal dehydrogenases, the quinone redox state is very low. The quinone redox state was thus very different when the electrons were provided by the external dehydrogenases compared to the internal dehydrogenases.

When a combination of substrates is used, the quinone redox state is set by the respiratory substrate that has the highest quinone redox state (Fig. 5). This shows that the quinones do not have a homogenous kinetic behavior, since for a comparable respiratory rate the quinone redox state can be very different. Fig. 5 shows that the relationship between quinone redox state and JO_2 exhibits to two different curves that are clearly linked to the origin of the electrons: there is one curve for the internal dehydrogenases and a distinct one for the external dehydrogenases. When a combination of substrates coming from both internal and external dehydrogenases is used, one can clearly see that the quinone redox state is (i) on the left-shifted curve when the electron right of way is from the external dehydrogenases and (ii) on the right-shifted curve when the electron right of way is from the internal dehydrogenases. This result indicates that there are two distinct quinone pools and that the competition process might depend on the existence of these pools.

4. Conclusion

We have previously shown that, in non-phosphorylating conditions, there is a competition for electrons to enter the respiratory chain [12,13]. In this paper, we show that this competition process applies to phosphorylating conditions, which are more physiological. Moreover the competition process is comparable whether it is assessed in state 4 or in state 3. The main process generated through this competition is a priority for cytosolic NADH reoxidation. Moreover, previous work from our laboratory has shown that NADH produced by cytosolic dehydrogenases is channeled through the mitochondrial porin to the respiratory chain via external NADH



Scheme 1.

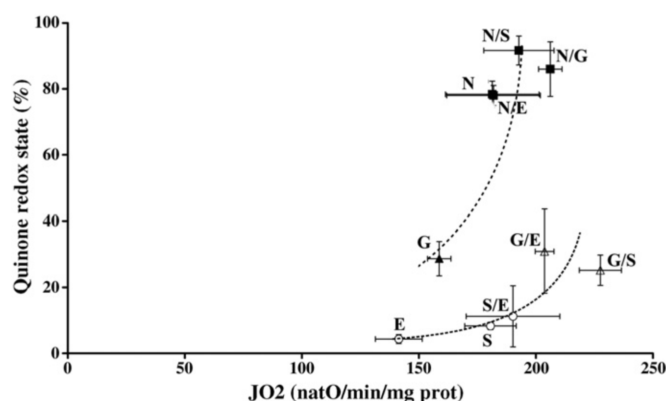


Fig. 5. Two distinct relationships between the quinone redox state and the respiratory rate under non-phosphorylating conditions suggesting the existence of two quinone pools involved in electron priority. Isolated mitochondria were incubated (0.5 mg ml^{-1}) in the mitochondrial buffer (see Materials and methods). Oxygen consumption (natO min^{-1} mg^{-1} protein) was measured at 28 °C with a Clark electrode. Quinone redox state was measured as described in the Materials and methods section. Subtrates were as follows: (N) NADH 1.5 mM, (E) Ethanol 1%, (G) G3P 20 mM, (S) succinate 10 mM. Results are means ± SD of at least three independent experiments.

dehydrogenases [9,10].In conclusion, cytosolic NADH reoxidation is mandatory for growth and such a priority has a relevant physiological meaning.

Since the mitochondrial respiratory chain has been shown to be organized into supramolecular complexes, we hypothesized that such an organization could be involved in the electron competition processes. However, from our data on mitochondria isolated from either a strain with altered electron competition or a strain with altered supramolecular complex organization, there does not seem to be any relationship between these processes in yeast mitochondria. In contrast, there seems to be a tight relationship between the quinone redox state and the electron competition process. Indeed, there are two distinct relationships between the respiratory rate and the quinone redox state that seem to indicate two quinone pools that are involved in the electron priority. The more reduced pool would be associated to the electron priority for the external dehydrogenases whereas the less reduced pool would be associated to the electron priority for the internal dehydrogenases.

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