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CATION MOVEMENTS AND RESPIRATORY RESPONSE IN YEAST MITOCHONDRIA TREATED WITH HIGH Ca^{2+} CONCENTRATIONS

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SUMMARY

1. Reinvestigation of the interaction of Ca^{2+} with mitochondria isolated from the yeasts *Saccharomyces cerevisiae* and *Candida utilis* has shown that respiration-linked cation uptake can be induced by high Ca^{2+} concentrations, in the range of 1–10 mM.

2. High Ca^{2+} concentration induces H^+ ejection, 2-fold respiratory stimulation, and an increase in the steady-state oxidation level of cytochrome *b*.

3. Ca^{2+} uptake by yeast mitochondria was demonstrated by spectrophometric recording of changes in calcium–murexide absorption.

4. Both Ca^{2+} uptake and proton ejection are strongly inhibited by uncouplers and by the electron transport inhibitor, antimycin A.

5. Inorganic phosphate stimulates the initial Ca^{2+} uptake rate about 8-fold.

6. No appreciable ATP-driven Ca^{2+} uptake could be detected under conditions suitable for respiration-linked transport.

7. Respiration-linked cation transport by yeast mitochondria has a narrow specificity, similar to that of liver mitochondria; Ca^{2+}, Sr^{2+} and Mn^{2+} are active, while Mg^{2+} and Na^+ are not.

8. Although isolated yeast mitochondria have the capacity for specific, respiration-linked Ca^{2+} uptake, this is probably of little physiological significance, since an efficient, high affinity Ca^{2+} transport system is lacking.

INTRODUCTION

A survey study of mitochondria isolated from a wide variety of organisms has revealed two types of organelles, based on their response to added Ca^{2+} (ref. 1). The first group, comprised of mitochondria from a large variety of vertebrate tissues,

Abbreviations: HEPES, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; EGTA, ethylene glycol-(β-aminoethyl ether)-*N,N'*-tetraacetic acid; FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone.

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is characterized by an extremely high affinity for Ca^{2+} and a rapid respiratory response which is accompanied by rapid cation uptake and proton ejection. Organelles of the second type exhibit low affinity for Ca^{2+}, while respiratory response to Ca^{2+} and ion transport may be slow or essentially absent. Notable in this second group are flight-muscle mitochondria from blowfly, in which Ca^{2+} uptake requires added phosphate[1,2], and yeast mitochondria[1,3] which do not exhibit appreciable Ca^{2+} uptake or respiratory response at cation concentrations as high as 0.13 mM[3]. The existence of these two types of mitochondria has been cited as supporting the view that vertebrate mitochoncria contain a specific carrier system for Ca^{2+} (ref. 4).

That yeast mitochondria may possess the electrochemical capacity necessary for energy-linked Ca^{2+} transport was indicated by our previous demonstration that respiration may be activated by K^+ in the presence of valinomycin[3]. We therefore suggested that it might be possible to overcome the apparent lack of a natural Ca^{2+} carrier and to trigger an energy-linked transport mechanism by using very high concentrations of Ca^{2+}. The present report demonstrates that yeast mitochondria prepared from *Saccharomyces cerevisiae* and *Candida utilis* can indeed carry out respiration-linked ion uptake when the medium Ca^{2+} is in the range of 1–10 mM.

MATERIALS AND METHODS

Growth of yeast

Saccharomyces cerevisiae, strain D261, was grown aerobically to stationary phase in natural media containing 3% ethanol or 2% dextrose as primary carbon source[5,6]. *Candida utilis*, was grown in a synthetic culture medium similar to that of Galzy and Slonimski, as modified by Ohnishi and Chance[7]. Ethanol (1.5%) served as primary carbon source and the initial iron concentration of the medium was less than 1.1 μM.

Preparation of mitochondria

Mitochondria from both strains were prepared by the method of Beck *et al.*[5], which involves partial digestion of cell walls by snail gut enzyme followed by homogenization in a colloid mill. Mitochondria were used immediately after isolation, or, in the case of *S. cerevisiae* mitochondria, stored intact under liquid nitrogen and rapidly thawed before use, as previously described[8]. In preparing *C. utilis* mitochondria no EDTA was used in either isolation or reaction media.

Analytical and spectral measurements

Respiration was measured with a Clark oxygen electrode as previously described[9]. In some experiments, simultaneous measurements of pH, oxygen and cytochrome *b* were performed: a Beckman Expandomatic pH meter with a combination pH electrode connected to a 10 mV strip chart recorder was used. Cytochrome *b* measurements were made with a dual wavelength spectrophotometer. In studies with *C. utilis*, an Aminco-Chance dual beam spectrophotometer was used: with *S. cerevisiae*, a spectrophotomer built by W. X. Balcavage was employed. Proton ejection experiments were initiated by introducing mitochondria into the reaction chamber which contained 3.0 ml of a reaction medium composed of 0.6 M mannitol, 4 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES)

buffer, pH 6.4, 5 mM sodium succinate, and varying amounts of Ca^{2+} or other reagents as described below. The H^+ ejection in each experiment was determined quantitatively by back-titration of the reaction mixture with standard NaOH or HCl.

Ca^{2+} uptake was measured kinetically by the murexide technique[10–12]. The calcium–murexide complex, as compared with murexide alone, exhibits a lower light absorbance at 540 nm and a higher absorbance at 470 nm with an isosbestic point at 507 nm. For this reason our experiments were carried out with the aid of a dual wavelength spectrophotometer equipped with an automatic reference light regulator[13] using 535 nm as a measurement wavelength and 504 nm as reference. These two wavelengths were chosen to allow maximum changes in absorbance without interference from other absorbing components of yeast mitochondria and were obtained employing 1.5 nm bandwidth filters (from Omega Opt. Co., Brattleboro, Vt.). In the presence of murexide, Ca^{2+} uptake by mitochondria (or Ca^{2+} binding by chelating agents) produces an increase in light absorbance at 540 (535) nm by reducing the concentration of Ca^{2+} available for formation of calcium–murexide complex.

Reagents

Crystalline sodium ATP (Lot 100C-7620), containing a Ca^{2+} concentration of less than 24 ppm, and ethylene glycol-(β-aminoethyl ether)-*N,N'*-tetraacetic acid (EGTA) were purchased from Sigma, St. Louis, Mo.; murexide (ammonium purpurate) from K and K Chemicals, Plainview, N.Y. Carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (FCCP) was a gift from Dr P. G. Heytler, DuPont Co., Wilmington, Del. Reagent grade $CaCO_3$ (98% $CaCO_3$) and $CaCl_2$ were obtained from Baker Chemical Co. Other reagents were as described previously[9].

RESULTS

H^+ ejection induced by divalent cations

The first indication that high Ca^{2+} concentrations could initiate cation movements in yeast mitochondria came from the observation that H^+ ejection from *S. cerevisiae* organelles was enhanced by Ca^{2+} as shown in Fig. 1. Our previous study[3] had shown that 0.133 mM Ca^{2+} caused no significant pH change, but as the figure shows, 2.5 mM Ca^{2+} significantly increased both rate and quantity of H^+ ejection. Fig. 2 illustrates the dependency of H^+ ejection on Ca^{2+} concentration. Although the accuracy of H^+ ejection rate measurements at low Ca^{2+} concentrations is somewhat decreased by the buffer, it was included to ensure maximum stability of mitochondria. Half-maximal stimulation of the initial ejection rate is obtained at approx. 1.5 mM Ca^{2+}, and to obtain maximal stimulation, 3–5 mM is required. In rat liver mitochondria maximal stimulation of respiration requires 20–30 times less Ca^{2+}, indicating a much higher affinity[14,15]. When other ions were tested for their ability to stimulate H^+ ejection, a pattern of specificity like that of rat liver mitochondria[14,16] was found (Table I). Mn^{2+} and Sr^{2+}, were at least as effective as Ca^{2+}, but neither Mg^{2+} nor the monovalent cation, Na^+, had a significant effect.

Stimulation of respiration by Ca^{2+}

Another attribute of Ca^{2+} interaction with mitochondria is the activation of respiration. As shown in Fig. 3, Ca^{2+} stimulates respiration of *C. utilis* mitochondria

oxidizing ethanol more than 2-fold. The Ca^{2+}-stimulated rate is slightly greater than that obtained with ADP (State 3). The cation concentration required for maximum stimulation falls in the range of 3–7 mM, depending on the mitochondrial preparation,

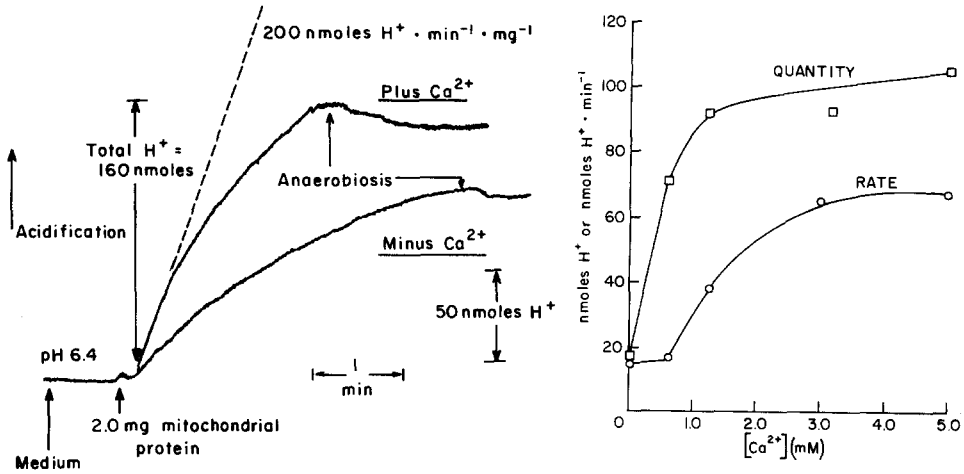

Fig. 1. Kinetics of Ca^{2+}-stimulated H^+ ejection by *S. cerevisiae* mitochondria. 2 mg of mitochondrial protein were added to a medium containing 5 mM sodium succinate, 4 mM HEPES buffer (pH 6.4), 0.6 M mannitol, ± 2.5 mM $CaCl_2$. The initial rate and total quantity of H^+ ejection are calculated as shown with added Ca^{2+}. Calibration of H^+ ejection (not shown) was determined by back-titration with standard NaOH. Temperature was 25 °C.

Fig. 2. Effect of Ca^{2+} concentration on H^+ ejection. *S. cerevisiae* mitochondria (1.6 mg protein) were added to 2.0 ml of a reaction medium containing 0.6 M mannitol, 4 mM HEPES buffer, 5 mM sodium succinate and Ca^{2+} as indicated. Temperature was 25 °C.

TABLE I

CATION DEPENDENCY OF H^+ EJECTION

Reaction medium, as described in Materials and Methods, was supplemented with various ionic constituents as indicated. The reaction was initiated by adding 2.4 mg of mitochondrial protein to a 2-ml, closed Lucite vessel in which a combination pH electrode was mounted. The H^+ ejection represents the quantity of H^+ produced during the consumption of 380 nmoles of O_2. The respiratory substrate was 6 mM succinate.

Cation added	*Concn (mM)*	*Total H^+ ejection (nmoles)*
none		115
NaCl	5	105
$MgCl_2$	2.5	105
$CaCO_3$	2.5	155
$MnCl_2$	2.5	160
$SrCl_2$	2.5	190

indicating that *C. utilis* and *S. cerevisiae* have similar low affinities for Ca^{2+}. If EGTA or EDTA is added to the Ca^{2+}-stimulated system, respiration rate falls to the state 4 level. Ca^{2+} also produced similar respiratory stimulation when either pyruvate *plus*

malate or glycerol 1-phosphate served as substrate. Once initiated, both respiration (Fig. 3) and H^+ ejection (Fig. 1) continue until anaerobiosis is attained. For this reason it is not possible to determine readily the stoichiometry of $Ca^{2+}/2e^-$ or H^+/Ca^{2+} ratios.

Ca^{2+}-induced oxidation of cytochrome *b* has been used as a sensitive indicator of Ca^{2+} interaction with the respiratory chain[17]. Provided sufficient Ca^{2+} is used,

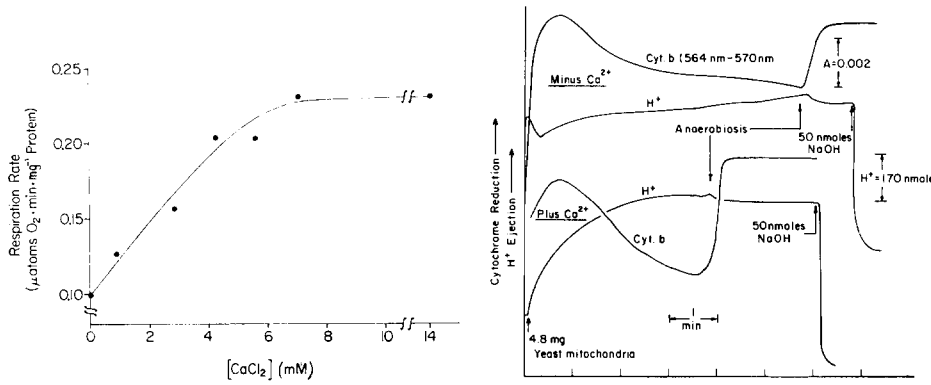

Fig. 3. Effect of Ca^{2+} on the ethanol oxidation by *C. utilis* mitochondria. Respiration was measured with a Clark oxygen electrode in a 2.5 ml vessel at 25 °C. The reaction mixture contained 0.6 M mannitol, 10 mM potassium phosphate buffer (pH 6.8) and 10 mM Tris–maleate buffer (pH 6.8). Ethanol (17 mM) served as a substrate in the presence of semicarbazide (10 mM). Mitochondrial protein concentration was 1.0 mg per ml of reaction mixture. The respiratory rate in the presence of added ADP was 0.2 μatom oxygen·min^{-1}·mg^{-1} protein.

Fig. 4. Effect of Ca^{2+} on redox levels of cytochrome *b* in *S. cerevisiae* mitochondria. Mitochondria (4.8 mg protein) were added to 5.0 ml of reaction medium containing 0.6 M mannitol, 4 mM HEPES buffer, 3.3 mM sodium succinate and, when present, 3 mM Ca^{2+}. The spectrophotometer cuvette contained a Clark oxygen electrode and a pH electrode. The vessel was continuously stirred with a small magnetic stirring bar. Temperature was 25 °C.

mitochondria from both types of yeast also display this type of interaction. In the experiment illustrated in Fig. 4, *S. cerevisiae* mitochondria were added to a system containing succinate with or without 3.0 mM Ca^{2+}. As indicated by the much larger absorbance change occurring at anaerobiosis, cytochrome *b* was considerably more oxidized when Ca^{2+} was present. As in previous experiments (Figs 1 and 2), Ca^{2+} also enhanced proton ejection. Although in the usual procedure for detecting Ca^{2+}-induced changes in animal mitochondria a pulse of Ca^{2+} is added to a suspension of mitochondria, we found it necessary with *S. cerevisiae* organelles to add the mitochondria to the Ca^{2+} solution in order to obtain reproducible results (Figs 1, 2, 4, 6 and 7). These difficulties, of undetermined origin, were not encountered with *C. utilis* mitochondria (Fig. 3 and Fig. 5).

Fig. 5 shows that Ca^{2+} also has an effect on cytochrome *b* oxidation in *C. utilis* mitochondria oxidizing pyruvate *plus* malate. In this case it was possible to add Ca^{2+} to a suspension of mitochondria already oxidizing substrate. The added Ca^{2+} induced an immediate rapid oxidation of cytochrome *b*, followed by a slower,

further oxidation. As with Ca^{2+}-stimulated oxygen uptake, the effect of Ca^{2+} on the steady state redox level of cytochrome *b* is completely reversed by EDTA. It may be noted that ADP addition causes a similar shift in cytochrome *b* oxidation level, but does not exhibit the slow, second oxidation phase obtained with Ca^{2+}. The uncoupler, 1799, causes a much greater shift in the oxidation level of the cytochrome than is caused by either ADP or Ca^{2+}.

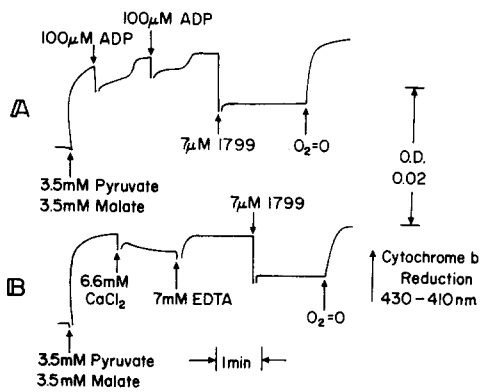

Fig. 5. Redox states of *C. utilis* mitochondrial cytochrome *b* in various metabolic states. (A) Redox changes of cytochrome *b* after addition of ADP or uncoupler 1799 during the oxidation of pyruvate *plus* malate. (B) Effect of Ca^{2+} on the redox states of cytochrome *b*. Reaction medium was the same as described in the legend of Fig. 3. Mitochondrial protein concentration was 2.2 mg per ml of reaction mixture. Other additions are shown in the figure.

Uptake of Ca^{2+} by mitochondria

Since proton ejection and respiratory effects are only indirect indicators of mitochondrial Ca^{2+} uptake, direct binding (uptake) studies were undertaken. Preliminary experiments with $^{45}Ca^{2+}$ indicated that some uptake was occurring, but the high Ca^{2+} concentrations required make such experiments technically difficult. Therefore, an alternative means of studying Ca^{2+} uptake using murexide[10–12] was employed. This method employs a dual wavelength spectrophotometer and permits one to measure the kinetics of Ca^{2+} uptake by following absorbance changes, using the wavelength pair 535 and 504 nm. Uptake of Ca^{2+} by mitochondria enhances absorbance at 535 nm by decreasing the concentration of cation available in the medium for formation of the calcium–murexide complex. Free murexide absorbs more strongly than the complex.

In order to obtain reasonable sensitivity, a Ca^{2+} concentration of 1.0 mM was employed. Although this is well below the Ca^{2+} concentration required to obtain maximal respiratory effects, it is quite adequate for measuring uptake by yeast mitochondria, as shown in Fig. 6 and Fig. 7. Fig. 6A shows that addition of *S. cerevisiae* mitochondria to a buffered medium containing murexide and succinate produces a relatively slow increase in absorbance at 535 nm, indicating that Ca^{2+} is being taken up by mitochondria, thus increasing the concentration of free murexide. The initial rate of uptake is about 11 nmoles/min per mg protein. This may be compared to the rate of H^+ ejection at the same Ca^{2+} concentration from Fig. 2. This H^+ ejection

rate is approx. 8 nmoles/min per mg protein, corrected for the rate of proton ejection in the absence of Ca^{2+}. This result suggests that the initial H^+/Ca^{2+} ratio may be near unity.

Fig. 7A shows that addition of 10 mM phosphate causes a 7- to 8-fold stimulation of the initial rate of Ca^{2+} uptake. However, after an initial rapid uptake, the rate decreases to the rate obtained withhout phosphate. Marked stimulation of Ca^{2+}

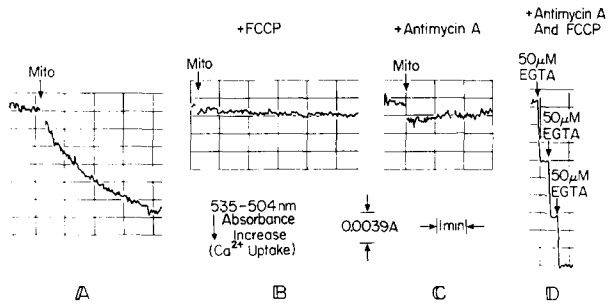

Fig. 6. Spectrophotometric measurements of Ca^{2+} uptake by *S. cerevisiae* mitochondria under different metabolic conditions. The reaction mixture contained 0.6 M mannitol, 10 mM potassium phosphate, 10 mM Tris–maleate, 6 mM sodium succinate, 1 μM $CaCl_2$ and 35 μM murexide. The pH of the mixture was 6.8 and the temperature 22 °C. This mixture was supplemented with 3 μM FCCP and 15 μg antimycin A/ml in D. The reaction was initiated with the addition of mitochondria (2.18 mg/ml) and changes in turbidity of the reaction mixture due to the addition of mitochondria were abolished by the automatic reference light compensator.

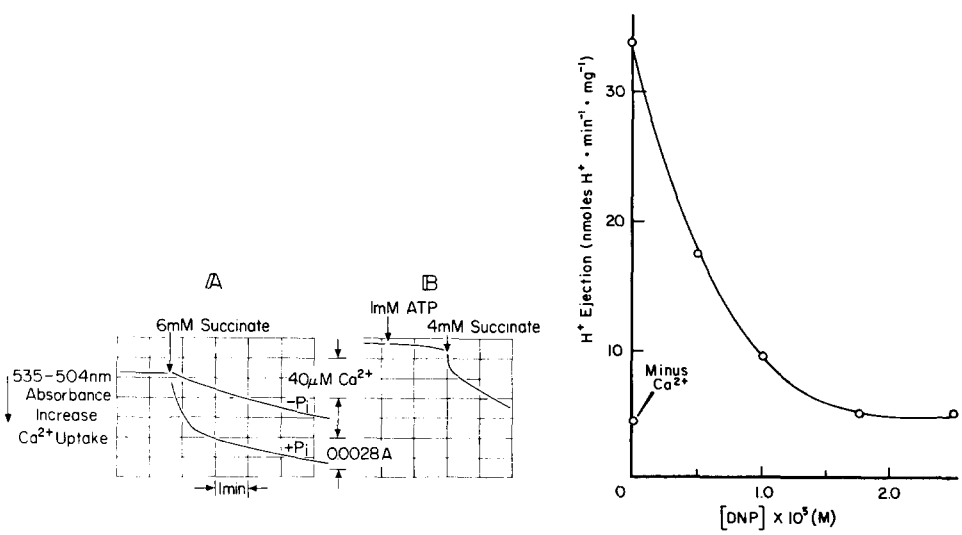

Fig. 7. Effects of phosphate and ATP on Ca^{2+} uptake by *S. cerevisiae* mitochondria. (A) The reaction mixture contained 0.6 mannitol, 10 mM Tris–maleate (pH 6.8), 1 mM $CaCl_2$, 2 mM $MgCl_2$ and 2.7 mg mitochondrial protein/ml. 10 mM potassium phosphate was present where indicated. (B) The reaction mixture contained 0.6 M mannitol, 10 mM Tris–maleate, 1 mM $CaCl_2$, 2 mM $MgCl_2$ and 2.7 mg protein/ml.

Fig. 8. The effect of 2,4-dinitrophenol (DNP) on Ca^{2+}-stimulated H^+ ejection by *S. cerevisiae* mitochondria. Conditions are as described for Fig. 1 except that Ca^{2+} concentration was 6 mM.

uptake by phosphate is characteristic of several other types of mitochondria, including those isolated from blowfly[2], rabbit spleen[1] and maize seedlings[18].

Association of Ca^{2+} uptake and H^+ ejection with energy metabolism

An intimate association of Ca^{2+} uptake with energy metabolism is indicated by the effects of uncouplers and inhibitors of respiration. Fig. 8 shows that Ca^{2+}-linked H^+ ejection is completely abolished by $2 \cdot 10^{-5}$ M 2,4-dinitrophenol. Similar effects of uncoupler are seen when Ca^{2+} uptake is measured spectrophotometrically with murexide, as indicated in Fig. 6B. The uncoupler, FCCP, abolishes completely the absorbance increase caused by Ca^{2+} uptake. Similarly, inhibition of electron transport by antimycin A inhibits Ca^{2+} uptake almost completely (Fig. 6C).

The data in Fig. 7B indicate that *S. cerevisiae* mitochondria, unlike intact liver mitochondria, do not accumulate appreciable Ca^{2+} in an ATP-driven process, but require a respiratory substrate. It should be emphasized that sufficient Mg^{2+} ion (2 mM) is present to prevent appreciable formation of interfering calcium–ATP complex. The sodium ATP employed contained less than 24 ppm Ca^{2+} contamination. Subsequent addition of succinate to the system induced a relatively rapid uptake of Ca^{2+}, probably due, in part at least, to inorganic phosphate released from ATP through ATPase action.

DISCUSSION

The results presented above establish that mitochondria isolated from two types yeast, *S. cerevisiae* and *C. utilis*, are capable of energy-linked uptake of Ca^{2+}, provided the Ca^{2+} concentration used is in the millimolar range. Direct evidence for Ca^{2+} uptake has been obtained by following disappearance of Ca^{2+} from the extramitochondrial medium by measuring changes in calcium–murexide absorption. The following characteristic phenomena associated with Ca^{2+} transport in vertebrate mitochondria[1] have also been observed with yeast mitochondria; (a) Ca^{2+}-induced proton ejection, (b) stimulation of oxygen uptake rate by added Ca^{2+} and (c) a Ca^{2+}-induced oxidation of cytochrome *b*. As in animal mitochondria, uncouplers and the respiratory chain inhibitor, antimycin A, prevent Ca^{2+} transport. Finally, Ca^{2+} transport by yeast mitochondria is enhanced by inorganic phosphate, as it is in mitochondria isolated from vertebrate tissues[1], blowfly flight muscle[2] and plants[18]. In yeast mitochondria this enhancement appears to be transient.

These findings, together with our previous observation that respiration in yeast mitochondria is activated by K^+ in the presence of valinomycin[3], are consistent with the existence of a respiration-linked electrochemical potential across the yeast mitochondrial membrane. As we suggested earlier[3], this potential can be expressed as Ca^{2+} transport, provided the ion concentration is sufficient to cause some movement across the membrane.

The present experiments do not permit us to make a firm decision on the mechanism by which high Ca^{2+} concentration triggers transport and the accompanying respiratory effects. The relatively narrow specificity of the cation transport system of liver mitochondria suggests the existence of a specific Ca^{2+} carrier[4]. In this respect yeast mitochondria resemble liver mitochondria, since Sr^{2+} and Mn^{2+} induce proton ejection, but Mg^{2+} and Na^+ do not. In mitochondria which have a high affinity for

Ca^{2+} it is tempting to assume that the specificity and the high affinity are attributes of the same molecular entity. However, in yeast mitochondria, where the apparent affinity for Ca^{2+} is low, the alternative explanation that cation specificity resides in a respiratory chain component remains a distinct possibility. Specific interaction with the respiratory chain does not necessarily require that the interaction be stoichiometric. If Ca^{2+} is sequestered within the mitochondrion as a soluble salt of phosphate or some other anion, it may specifically activate the respiratory chain when it reaches a certain threshold concentration. This assumes, of course, that the intramitochondrial pH is not so high as to render calcium phosphate insoluble. The consequent ejection of H^+ may now produce a gradient which can drive additional Ca^{2+} uptake. According to this scheme, Ca^{2+} serves mainly as a specific activator of respiration: the stoichiometry of uptake is determined by whatever anions are available in the system. In the absence of added phosphate, activation of respiration by Ca^{2+} may or may not be observed depending on (a) the concentration of endogenous anions and substrate anions, (b) the dissociation constants and solubility of the Ca^{2+} salts of these anions, (c) the intrinsic permeability of the inner membrane to Ca^{2+} and various anions, (d) intra- and extramitochondrial pH, (e) the extramitochondrial Ca^{2+} concentration, and (f) the rate of Ca^{2+} uptake. According to this idea, blowfly mitochondria, which are highly impermeable to most anions[19], cannot attain the critical, "activating" Ca^{2+} concentration within the mitochondrion in the absence of added permeant anion such as phosphate. Vertebrate mitochondria, on the other hand, appear to be extremely permeable to Ca^{2+}, probably because they contain a specific Ca^{2+} transfer system. Therefore, "activation" of the respiratory chain may occur at very low Ca^{2+} concentrations in these mitochondria, usually even in the absence of added phosphate.

Another way of rationalizing the apparent cation specificity of yeast mitochondria is to assume that Ca^{2+} transport in vertebrate mitochondria involves two membrane components, one responsible for ion recognition, and another for rapid translocation. According to this idea, yeast mitochondria may contain only the first component. It is also possible that the translocation component has been inactivated during the preparation of yeast mitochondria.

While the initial events described here resemble in many respects those seen when Ca^{2+} is taken up by animal mitochondria, the two systems are difficult to compare because extramitochondrial Ca^{2+} concentration is very high throughout the experiment. The continuous presence of this high level of Ca^{2+} may be responsible for the fact that both H^+ ejection and rapid respiration continue until anaerobiosis is attained.

The apparent lack of appreciable ATP-driven Ca^{2+} uptake in yeast mitochondria represents a significant difference from animal mitochondria[20]. Whether this indicates a fundamental difference in organization of the energy-coupling systems of the two types of mitochondria, or simply reflects the use of unsuitable experimental conditions, has not been determined. Several previous observations may be pertinent; (a) Yeast mitochondria suspensions, as ordinarily prepared, contain significant non-specific Mg^{2+}-activated ATPase activity, which is quite active in the pH range used in this study[21]. (b) The pH optimum for oligomycin-sensitive ATPase from yeast mitochondria is high, about 9 (ref. 22). (c) Yeast mitochondria display little or no 2,4-dinitrophenol-stimulated ATPase[22]. (d) Energy-dependent reversal of

electron transport in yeast mitochondria can be driven by "internally" generated increase in phosphate potential, but not by increase in external ATP[23]. (e) Similarly, a change in midpoint potential of yeast cytochrome by cannot be induced by adding extramitochondrial ATP[23]. These observations all indicate that yeast mitochondrial ATPase is relatively inaccessible to external ATP. It may also be noted that Pedersen and Coty[24] have shown that inner membrane vesicles prepared from rat liver mitochondria can support respiration-dependent Ca^{2+} uptake, but have lost the ability to support uptake linked to ATP hydrolysis. This indicates that coupling of various energy-linked functions is subject to selective change, probably as a function of membrane conformation. Apparently the natural conformation of the yeast mitochondrial membrane is highly favorable for ATP formation, but not for ATP-linked, intramitochondrial processes, including Ca^{2+} transport.

The demonstration of energy-linked Ca^{2+} uptake by yeast mitochondria is of potential importance for future studies. For example, yeast mitochondria might be used to assay for Ca^{2+}-transferring activity of Ca^{2+}-binding factors isolated from animal mitochondria[25,26]. Extracts of yeast mitochondria might also be useful in studies of isolated factors thought to be associated with Ca^{2+} transport, since comparable yeast factors should be lacking, or possess properties distinctly different from factors prepared from animal mitochondria. Finally, Ca^{2+} transport studies may provide a new dimension in investigations involving genetic modification of yeast mitochondria[5,6,27].

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