Biochimica et Biophysica Acta, 357 (1974) 453—456

BBA Report

BBA 41260

BONGKREKIC ACID SENSITIVITY OF RESPIRATION-DEFICIENT MUTANTS AND OF PETITE-NEGATIVE SPECIES OF YEASTS

J. ŠUBÍK [a], J. KOLAROV [b] and L. KOVÁČ [c]

[a] *Food Research Institute, Bratislava,* [b] *Institute of Experimental Oncology, Slovak Academy of Sciences, Bratislava and* [c] *Psychiatric Hospital, Pezinok (Czechoslovakia)*

(Received June 26th, 1974)

Summary

1. Growth on glucose of cytoplasmic respiration-deficient (ρ^-) mutants isolated from five strains of *Saccharomyces cerevisiae* and one strain of *Saccharomyces carlsbergensis* were arrested by the inhibitor of mitochondrial adenine nucleotide translocation, bongkrekic acid. This indicates that the mitochondrial adenine nucleotide translocation system is preserved and necessary for growth in a number of independent ρ^- mutants.

2. Growth of three "petite-negative" yeast species was arrested by a combined inhibition of respiration by antimycin A and of adenine nucleotide translocation by bongkrekic acid. Thus, the arrest of growth upon inhibition of adenine nucleotide translocation in non-respiring cells is not specific for ρ^- mutants and may be a general characteristic of eucaryotic cells.

The system translocating adenine nucleotides across the inner mitochondrial membrane has entered into the focus of recent investigations of mitochondria, being a suitable model for elucidation of membrane transport phenomena [1, 2] and also because of the possibility of its intimate involvement in energy conservation [3] and in the control of cell growth [4]. An obligatory requirement of the mitochondrial adenine nucleotide translocation for normal functioning of eucaryotic cells has been postulated [4] and supported also by the finding that the translocation system is preserved intact in non-respiring mitochondria of a respiration-deficient ρ^- strain of *Saccharomyces cerevisiae* (ref. 5,and Kolarov, J. and Klingenberg, M., in preparation). However, the latter finding has become a matter of controversy [6, 7] and has been tentatively ascribed to the difference in strains employed by independent investigators [7].

TABLE I

EFFECT OF BONGKREKIC ACID ON "PETITE-NEGATIVE" SPECIES OF YEASTS

Characteristic	Species				
	E. magnusii	*Sch. pombe*	*Z. fermentati*	*C. parapsilosis*	*Sch. versatilis*
Growth yield (10^6 cells per ml) after 48 h growth on 0.5 % glucose in the presence of the following inhibitors:					
None	230	36	190	400	8
Antimycin A (2 μg/ml)	< 1	28	46	240	3
Bongkrekic acid (8.8 μM)	2	16	48	130	0
Antimycin A + Bongkrekic acid	< 1	11	11	12	0
Inhibition of respiration (%) by bongkrekic acid (18 μM)	81	71	29	82	*

*This strain is non-respiring.

In the present work, bongkrekic acid, a specific inhibitor of mitochondrial adenine nucleotide translocation in isolated mitochondria [8, 9] and in intact yeast cells [4] has been employed to show the presence of the adenine nucleotide translocation system in a number of respiration-deficient mutants and also the necessity of the system for growth of different species of yeasts.

The following wild-type strains of *S. cerevisiae* were used: DT XII, $H3CR_1 x H6CR_2$, D-225-5A, DPI-1B, 55 R5 3C 11, IL 8-8D. The four last strains were from the collection of Centre de génétique moléculaire du CNRS, Gif-sur-Yvette. From these strains, and also from *Saccharomyces carlsbergensis* NCYC 74, respiration-deficient mutants were prepared by prolonged growth in the presence of ethidium bromide [10, 11]. The following yeast species were obtained from the yeast collection of Chemical Institute of SAV, Bratislava: *Endomyces magnusii* 42-1-1, *Schizosaccharomyces pombe* 44-1-3, *Schizosaccharomyces versatilis* 44-3-1, *Zygosaccharomyces fermentati* 35-8-2, and *Candida parapsilosis* var. *intermedia* 29-20-10. The cells were cultured aerobically in a semi-synthetic medium with glucose as carbon source on a reciprocal shaker at 30 °C. Growth was followed by counting the cells in a haemocyte chamber and by plating on solid agar media. Respiration-deficient mutants were detected by the tetrazolium-overlay method [12].

The five strains of *S. cerevisiae* and also the strain of *S. carlsbergensis* exhibited exactly the same properties as found previously with a single strain of *S. cerevisiae* DT XII [4]. The wild-type cells did grow on glucose in the presence of bongkrekic acid but with a lower yield than in the absence of the inhibitor using for growth energy furnished by fermentation. When the medium containing bongkrekic acid was also supplemented with antimycin A, so that the formation of ATP in mitochondria was prevented and no ATP could enter the mitochondria via the blocked adenine nucleotide translocation system, the cells were converted into respiration-deficient mutants. Thus, intramitochondrial ATP was required for normal replication of mitochondrial DNA in all the strains.

Respiration-deficient mutants, presumably containing no mitochondrial DNA [10, 11], prepared from the five strains of *S. cerevisiae* and also from *S. carlsbergensis* with ethidium bromide, could grow on glucose. However, in the presence of bongkrekic acid, only a few generations could be formed (range 2—4) and then the growth was entirely arrested and could only be restored if the cells were transferred into medium containing no bongkrekic acid. This indicates that in order to assure growth of all six strains of *Saccharomyces*, ATP must have been supplied to mitochondria via the mitochondrial adenine nucleotide translocation system once the cells were converted into non-respiring ρ^- mutants.

In order to find out whether the conversion into the respiration-deficient mutants is a prerequisite for growth arrest with bongkrekic acid, similar experiments were performed with "petite-negative" species of yeasts. These species do not form viable respiration-deficient mutants [13, 14]. The results are summarized in Table I.

As can be seen, growth of *E. magnusii* was arrested by single inhibition by antimycin A or by bongkrekic acid. This species apparently cannot obtain enough energy from fermentation so that the growth could not proceed once the supply of ATP by mitochondria was being inhibited. Similar behaviour was also observed with a number of mold species [15]. The growth yield on glucose of the other three species, *S. pombe* ,*Z. fermentati* and *C. parapsilosis*, was only reduced but not arrested by single inhibition by either antimycin A or bongkrekic acid, indicating that the growth could be sustained by the less efficient fermentation system. When both inhibitors were present, the growth ceased despite the fact that the supply of energy by fermentation had not been affected by the inhibitors. (Growth of *Z. fermentati* and *C. parapsilosis* continued at an extremely low rate after 48 h, so that, after another 48 h, an additional two generations of cells appeared. This was presumably due to a small leak along the paths inhibited by either antimycin A or bongkrekic acid.) Respiration-deficient mutants were induced by neither single inhibitors nor by their combination.

It is thus clear that if in *Saccharomyces* the conversion into respiration-deficient mutants must have taken place before growth was arrested by the combined inhibition with antimycin A and bongkrekic acid, a simple inhibition of respiration by antimycin A rather than complex mitochondrial deficiency which characterizes the cytoplasmic ρ^- mutation is sufficient to prevent growth of some other yeast species on glucose in the presence of bongkrekic acid.

This is also borne out by the results obtained with *S. versatilis*. This species was found to be non-respiring and to contain no cytochromes [16] and yet, contrary to the ρ^- mutants [17, 18] to exhibit the mitochondrial ATPase sensitive to oligomycin. As shown in Table I, a single inhibition by bongkrekic acid did prevent growth of this species on glucose.

Two general conclusions can be drawn from the present results:

(1) As judging from in vivo inhibition, the preservation of the mito-

chondrial adenine nucleotide translocation system is not the property of only one strain of *S. cerevisiae* studied previously [4] but apparently a universal characteristic of cytoplasmic respiration-deficient ρ^- mutants. This, of course, does not exclude the possibility that the membrane environment in which the translocator operates in mitochondria may affect some properties of the translocation system.

(2) The arrest of growth upon inhibition of mitochondrial adenine nucleotide translocation is not a particular property of the cytoplasmic ρ^- mutants. The necessity of the functional adenine nucleotide translocation system to assure continual growth of non-respiring cells even when energy requirements are fully covered from extramitochondrial sources may be a general property of eucaryotic organisms.

References

1 Klingenberg, M. and Buchholz, M. (1973) Eur. J. Biochem. 38, 346—358
2 Vignais, P.V., Vignais, P.M., Lauquin, G. and Morel, F. (1973) Biochimie 55, 763—778
3 Boyer, P.D. (1973) Int. Congr. Biochem. (Stockholm) Abstr. No. 211
4 Šubík, J., Kolarov, J. and Kováč, L. (1972) Biochem. Biophys. Res. Commun. 49, 192—198
5 Kolarov, J., Šubík, J. and Kováč, L. (1972) Biochim. Biophys. Acta 267, 457—464
6 Perkins, M., Haslam, J.M. and Linnane, A.W. (1972) FEBS Lett. 25, 271—274
7 Haslam, J.M., Perkins, M. and Linnane, A.W. (1973) Biochem. J. 134, 935—948
8 Henderson, P.J.F. and Lardy, H.A. (1970) J. Biol. Chem. 245, 1319—1326
9 Klingenberg, M., Grebe, K. and Heldt, H.W. (1970) Biochem. Biophys. Res. Commun. 39, 344—351
10 Goldring, E.S., Grossman, L.I., Krupnick, D., Creyer, D.R. and Marmur, J. (1970) J. Mol. Biol. 52, 323—335
11 Nagley. P. and Linnane, A.W. (1970) Biochem. Biophys. Res. Commun. 39, 986—996
12 Ogur, M., St. John, R. and Nagai, S. (1957) Science 125, 928
13 Bulder, C.J.E.A. (1964) Antonie van Leeuwenhoek J. Microbiol. Serol. 30, 442—454
14 DeDeken, R.H. (1966) J. Gen. Microbiol. 44, 157—165
15 Šubík, J. and Behúň, M. (1974) Arch. Mikrobiol. in the press
16 Wickerham, L.J. and Duprat, E. (1945) J. Bacteriol. 50, 597—607
17 Kováč, L. and Weissová, K. (1968) Biochim. Biophys. Acta 153, 55—59
18 Schatz, G. (1968) J. Biol. Chem. 243, 2192—2199