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Specific changes in nucleotide and lipid synthesis are linked to growth defects in intramitochondrial energy-depleted yeast cells

I. Hapala [a,*], B. Hunáková [a], G. Gerzaničová [a], P. Butko [b]

[a] *Institute of Animal Biochemistry and Genetics, Slovak Academy of Sciences, 900 28 Ivanka pri Dunaji, Slovak Republic*
[b] *Institute for Biological Sciences (M-54), NRC, Ottawa, Ont., K1A 0R6, Canada*

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Abstract

Energy depletion of yeast mitochondria caused immediate arrest of cell growth which could be partially reversed by enrichment of the medium. The analysis of radiolabel incorporation into nucleotides and lipids revealed significant changes in substances with possible links to mitochondrial activity (UMP, phosphatidyl-ethanolamine, cardiolipin, phosphatidylglycerol). Changes in sterol pattern indicated inhibition of squalene epoxidase located in endoplasmic reticulum. Although the results demonstrated severe impairment of anabolic processes in intramitochondrial energy-depleted cells, all major nucleotide and lipid species were significantly labelled in long-term experiments.

Key words: Energy depletion; Mitochondrion; Nucleotide synthesis; Phospholipid synthesis; Sterol synthesis; Cell growth; (*S. cerevisiae*)

Oxidative synthesis of ATP is the best known function of mitochondria in eukaryotic cells. However, mitochondria are involved in a number of cellular processes not directly linked to cellular energetics that can be studied in intact cells only. *Saccharomyces cerevisiae* is an organism particularly suitable for such studies. Respiratory deficient *rho*$^-$ mutants of this yeast grow well on glucose but their growth is arrested if the exchange of ATP and ADP between cytosol and mitochondria is inhibited by a specific inhibitor bongkrekic acid (BKA) or by mutation [1–3]. This growth arrest was explained by energy depletion of mitochondria causing defects in mitochondrial biogenesis [5,6] and/or cessation of synthetic processes in mitochondria [2,4]. Growth pattern of *rho*$^-$ cells in the presence of BKA is dependent on cultivation conditions (Fig. 1): the arrest is immediate in synthetic medium but it is partially reversed after supplementing the cells with peptone and yeast extract. The most plausible explanation for this effect is that peptone and yeast extract contain some substance(s) able to substitute for mitochondrial defects induced by energy depletion. The effect of yeast extract and peptone was dependent on the period of incubation of cells with BKA: no stimulation of growth was observed if this period exceeded 12 h (results not shown). This indicated that irreversible changes accumulated in intramitochondrial energy-depleted cells that could be linked to general defects in mitochondrial biogenesis [6].

Growth-promoting component of peptone and yeast extract could not be identified so far in spite of extensive efforts [4]. Similarly, no significant changes in the content of individual amino acids could be detected [4]. Other substances synthesized at least partially in mitochondria are nucleotides [7,8] and lipids (for review, see Ref. [9]). Therefore, the effect of intramitochondrial energy depletion on the synthesis of these substances was investigated in detail.

Differences in nucleotide levels between exponential and stationary-phase control cells (C-6 and C-24,

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[*] Corresponding author. Fax: +42 7 943932.

Abbreviations: BKA, bongkrekic acid; PL, phospholipids; CL, cardiolipin; lyso-PL, lysophospholipids, PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PS, phosphatidylserine; TLC, thin-layer chromatography.

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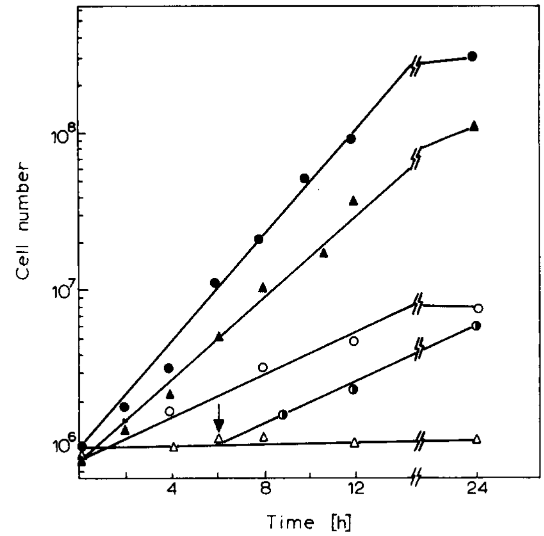

Fig. 1. Effect of bongkrekic acid on the growth of *rho*$^-$ cells. Wild-type diploid strain DTXIIA [*rho*$^-$] was grown on semisynthetic (circles) or synthetic (triangles) media (pH 5.0, 2% glucose) in the absence (closed symbols) or presence (open symbols) of BKA (final concentration 20 μM). After 5 h (arrow) peptone and yeast extract were added to an aliquot of cells treated with BKA in synthetic medium (half-closed circles).

Table 1) could be explained by exhaustion of glucose from the medium. The changes included reduced label in nucleotide triphosphates paralleled by increase in labelling of corresponding di- and monophosphates. An interesting observation was that nucleotide triphosphates were still present in significant amounts in *rho*$^-$ cells after 24 h of cultivation when glucose was not detectable in growth medium (results not shown). This was in contrast to previous reports on complete ATP depletion of stationary-phase *rho*$^-$ cells [10]. Several statistically significant changes in incorporation of radiolabel into individual nucleotides were observed between BKA-treated and corresponding control cells. Some of them were observed only in 24 h variants and could be attributed in part to higher energy resources in BKA-treated cells. Two of the changes – in GTP and UMP – were observed in cells treated with BKA for both 6 and 24 h and could thus be related specifically to energy depletion of mitochondria. While 1.6–2.9-fold increase in GTP is difficult to explain in terms of mitochondrial defects, 2.3–3.6-fold decrease in UMP could reflect a change in dihydroorotate dehydrogenase activity. This is a mitochondrial enzyme with activity linked to respiration in animal cells and in *Neurospora* [7,11]. The compartmentation of this enzyme in yeast is more complex. In *Schizosaccharomyces pombe* it is located in mitochondria but it is reported to be cytosolic in *S. cerevisiae* and its activity is independent on mitochondrial respiration in this organism [12].

Table 1
Effect of intramitochondrial energy depletion on nucleotide synthesis

Nucleotide	% of incorporated radioactivity			
	C-6	BKA-6	C-24	BKA-24
GTP	4.75 ± 0.92	7.63 ± 1.63 *	2.92 ± 0.49	8.49 ± 1.98 **
ATP	26.69 ± 2.85	30.84 ± 2.63	8.24 ± 1.77	35.99 ± 1.73**
UTP	8.18 ± 0.94	9.15 ± 2.24	3.30 ± 1.0	2.27 ± 0.72
CTP	3.45 ± 1.17	5.37 ± 1.08	2.32 ± 0.47	4.94 ± 1.05
GDP	1.83 ± 0.40	3.70 ± 1.75	3.09 ± 0.64	n.d.
ADP	6.98 ± 0.27	8.17 ± 1.03	11.82 ± 1.42	10.23 ± 2.42
UDP	2.89 ± 0.87	2.61 ± 0.42	4.11 ± 0.64	2.25 ± 0.51 *
CDP	3.48 ± 0.72	2.30 ± 0.57	3.11 ± 0.15	2.32 ± 1.02
GMP	n.d.	n.d.	n.d.	1.38 ± 0.19
AMP	3.05 ± 1.18	2.96 ± 1.14	13.51 ± 1.35	3.65 ± 0.91 **
UMP	12.89 ± 0.94	5.56 ± 1.64 **	8.36 ± 1.55	2.73 ± 0.78 **
CMP	1.54 ± 0.70	n.d.	0.64 ± 0.16	0.55 ± 0.21
NAD	13.54 ± 2.03	13.04 ± 2.87	26.56 ± 4.16	17.04 ± 2.90 **
cAMP	1.49 ± 0.10	1.03 ± 0.49	0.27 ± 0.13	1.10 ± 0.56

Cells of the wild-type *rho*$^-$ strain DTXIIA were labelled in phosphate-limited (final concentration 3 mM) YNB medium with 5% glucose and carrier-free $H_3^{32}PO_4$ (1 MBq/ml); final BKA concentration was 20 μM. Nucleotides were extracted after 6 and 24 h of incubation by a modified method of Weibel et al. [13] and separated by 2-D TLC [14]. Labelling of individual nucleotides was quantified by scintillation counting after autoradiography. Relative content of nucleotides was determined as the percentage of total radioactivity recovered from all nucleotide spots. The values are mean (± S.E.) of three independent experiments; statistical significance of differences between control and BKA-treated variants were evaluated by two-tailed *t*-test: * $P < 0.05$; ** $P < 0.01$; n.d., not detectable.

In order to discriminate the effect of residual growth on lipid synthesis, radioactive label ($H_3^{32}PO_4$ or ^{14}C-acetate) was added either simultaneously with or 6 h after the addition of BKA. Changes in labelling pattern of phospholipids and neutral lipids were always more apparent in the latter case which could be explained by the accumulation of defects in energy-depleted mitochondria during prolonged incubation with BKA. Statistically significant changes in the incorporation of [^{32}P]phosphate into phospholipids (Table 2) included higher labelling of PI as well as reduced labelling of PE, PG and CL. The latter changes are consistent with subcellular distribution of enzymes for phospholipid synthesis in yeast since PS decarboxylase (major enzyme for PE synthesis in yeast) and enzymes for PG and CL synthesis are reported to be mitochondrial [9]. The molecular mechanism of these effects of mitochondrial energy depletion could include defects in enzyme delivery to mitochondria due to disturbed mitochondrial biogenesis [6] or (although less probable) more direct effects of intramitochondrial ATP depletion or membrane depolarization.

The most interesting changes in labelling pattern of neutral lipids (Table 3) indicated disturbed synthesis of sterols. Reduced labelling of sterols (both free and esterified) and accumulation of sterol precursor squalene are similar to the pattern observed in cells with squalene epoxidase inhibited by a specific inhibitor – terbinafine (Gerzaničová, G. and Hapala, I., unpublished results). This result was rather unexpected as squalene epoxidase is localized in endoplasmic reticu-

Table 2
Effect of intramitochondrial energy depletion on phospholipid synthesis

Lipid	% of incorporated activity		
	control	BKA-treated	
		label A	label B
Lyso-PL	5.86±0.76	6.56±0.27	4.87±0.28
PI	23.12±0.38	27.78±0.40 **	30.28±0.42 **
PS	8.36±0.14	7.33±0.23	8.86±0.12
PC	37.63±0.94	39.28±0.15	38.11±0.23
PE	18.33±0.36	13.53±0.11 **	12.96±0.03 **
PG	0.38±0.03	0.05±0.02 **	0.02±0.01 **
CL	1.73±0.03	0.25±0.05 **	0.05±0.05 **
PA	1.12±0.05	1.14±0.08	1.32±0.02
Unidentified	2.85±0.49	3.58±0.62	2.45±0.38

Strain W303-1B/4 genotype MAT *α ade2 leu2 his3 trp1 ura3* [*rho*^{0}] was grown in phosphate-limited semisynthetic medium as described in the legend of Table 1. $H_3^{32}PO_4$ (200 kBq/ml) was added either simultaneously (Label A) or 6 h after addition of BKA (Label B). Lipids were extracted by a modified method of Bligh and Dyer [15]. Separation of PL by 2-D TLC on silicagel plates was performed according to [16]. Label in individual phospholipid spots was determined as for nucleotides in Table 1. Values are means±S.E. from two or three independent determinations. Statistical significance of differences between control and BKA-treated cells was determined by two-tailed *t*-test: ** $P < 0.01$.

lum [9] and no spatial or functional links to mitochondria were reported for this enzyme so far. Indirect relation to mitochondrial activity is supported by the fact that (unlike to the situation for phospholipids) sterol synthesis proceeded undisturbed in the first hours after BKA addition (see the difference between variants A and B of BKA-treated cells) when mitochondria should be already energy depleted and the defects accumulated in the later periods of incubation with BKA. Quite interesting was the observation that the reduced labelling of free sterols was much more evident than that of esterified sterols. Sterol esters are considered to be a storage form of sterols in yeast and they are usually mobilized before any significant drop in free sterol concentration is visible. Different response of these two forms of sterols to intramitochondrial energy depletion could thus indicate a defect in sterol storage mobilization under these conditions.

Table 3
Effect of intramitochondrial energy depletion on neutral lipid synthesis

Lipid	% of incorporated activity		
	control	BKA-treated	
		label A	label B
Sterols	22.17±0.52	20.58±0.15	9.83±0.09 **
Fatty acids	6.64±0.04	5.01±0.10 *	4.74±0.05 *
Triglycerides	27.71±0.23	33.82±0.58 **	32.50±0.20 **
Sterol esters	24.75±0.60	20.01±1.37 *	14.67±1.39 **
Squalene	3.37±0.62	5.70±1.52	21.70±0.78 **
Unidentified	14.32±0.83	14.25±0.48	14.98±0.18

Strain W303-1B/4 [*rho*^{0}] was grown in YNB medium with 2% glucose. Addition of label (sodium [^{14}C]acetate, 55 kBq/ml) and extraction of lipids was as described in Table 2; separation of neutral lipids by 1-D TLC was performed according to [17]. Quantification of individual lipids and statistical evaluation were as described in Table 1. * $P < 0.05$, ** $P < 0.01$.

Our results demonstrate that energy depletion of yeast mitochondria causes a severe impairment of cellular metabolism of nucleotides, phospholipids and neutral lipids. However, observed changes can hardly be a single cause of immediate growth arrest after the addition of BKA since at least minimal labelling was observed in all nucleotide and lipid classes after prolonged incubation. At present we can only speculate if synergic effect of numerous smaller changes results in a general disturbance of cellular metabolism or whether a more specific signal from energy-depleted mitochondria is involved in the arrest of cellular growth.

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