Incorporation of ^{3}H-UTP into Mitochondria Isolated from Cytoplasmic and Nuclear Respiratory-Deficient Yeast Mutants

In yeast, a modification of mitochondrial DNA (M-DNA) leading to respiratory deficiency[1] ($\varrho^+ \rightarrow \varrho^-$) or phenotypically identical chromosomal mutations[2] ($P \rightarrow$ p) result in the impairment of protein synthesis by isolated mitochondria[3–5]. The existence and fundamental properties of DNA-dependent RNA synthesis in isolated wild-type yeast mitochondria have been well established[6–8]. Therefore, an attempt was made to elucide whether the absence of protein synthesis in isolated mutant mitochondria is due to the block at the transcriptional or translational level, by testing the incorporation of ^{3}H-UTP into a trichloroacetic acid-insoluble fraction of isolated mutant mitochondria.

Material and methods. Strains of *Saccharomyces cerevisiae* used in the study are listed in the Table. Nuclear gene (p) mutants S 19, Z 5 and S 9×9 were prepared and kindly supplied by Dr. T. M. LACHOWICZ, Polish Academy of Sciences, Wróclaw (Poland). The fraction of double (pϱ^-) mutants in haploid cultures did not exceed 40% (ref. [5]). Other strains were kindly provided by Dr. H. JAKOB, Centre de Génétique Moléculaire, Gif-sur-Yvette (France). All cultures except S 9×9 were haploid. Cultivation of the cells and the preparation of spheroplasts and mitochondria were performed according to KOVÁČ et al.[9,10] Mitochondria were further purified as described previously[3]. Sterile solutions and vessels were used for the preparation of spheroplasts and mitochondria. The mitochondria (0.1 ml suspension of mitochondria in 0.65 *M* mannitol or sucrose-1 m*M* EDTA, pH 7.6, containing about 2 mg protein) were added to a 30 °C warm medium containing in 0.9 ml: 40 µmoles *Tris*-HCl (pH 7.4), 100 µmoles KCl, 100 µmoles sorbitol, 5 µmoles KH_2PO_4, 10 µmoles $MgCl_2$, 0.5 µmole $MnCl_2$, CTP, GTP (50 µmoles each), 5 µmoles phosphoenolpyruvate, 1 µmole ATP, 50 µg pyruvate kinase and 7.5 µC of ^{3}H-UTP (specific activity 1.5 C per mmole). During incubation at 30 °C, samples (0.1 ml) were taken at 0, 5, 10, 30 and 60 min and further treated as described by WINTERSBERGER[7]. Radioactivity was determined in a Packard Tri Carb scintillation counter with a counting efficiency of 10%. As detected by plating samples of the incubating medium with mitochondria on nutrient agar plates, number of viable bacteria present in 1 ml of the mixture was usually about 1000. DNA content in mitochondria was determined in hot $HClO_4$ extract[11] by the method of BURTON[12].

Results and discussion. Under the conditions described above mitochondria isolated from wild-type strain incorporated about 20 pmoles of ^{3}H-UTP per mg protein during 10 min incubation. This value corresponds to that found by SOUTH and MAHLER[8] under similar conditions. Mitochondria from all the mutant strains tested incorporated also significant amounts of ^{3}H-UTP into the trichloroacetic acid-insoluble fraction. Kinetics of incorporation into mitochondria from all the strains tested were similar to that described by WINTERSBERGER[7] for wild-type yeast mitochondria, e.g. time course of ^{3}H-UTP incorporation was linear within first 5–10 min of incubation. The incorporation was inhibited by actinomycin C (Table). No relation between the degree of supressivness of the ϱ^- strains and the rate of incorporation was found. The incorporating activity of mitochondria isolated from the same strain varied considerably in different experiments, so that a precise quantitative comparison of activities has not yet been possible. Contribution by contaminating nuclei and whole cells to the incorporation activity of mitochondria does not seem to be likely, because further purification of mitochondria by equilibrium centrifugation[13] did not result in a significant decrease of their incorporating activity or in a change in their DNA content. Incorporation of ^{3}H-UTP measured in the experiments thus probably reflects true mitochondrial RNA synthesis.

Existence of RNA synthesis in isolated mutant mitochondria is in agreement with recent findings of FUKUHARA et al.[14] who demonstrated in ϱ^- mutants the presence of RNA species which could hybridize with M-DNA. In addition, the finding that sedimentation profile of mitochondrial RNA from ϱ^- mutant differed from that of wild-type strain[15] has also indicated the possibility of the in vivo transcription of ϱ^- M-DNA.

Incorporation of ^{3}H-UTP into mitochondria isolated from wild-type and respiratory-deficient mutant yeast cells

Strain	Respiratory genotype	Degree of supressivness[a] (%)	pmoles of ^{3}H-UTP incorporated × mg^{-1} protein × 10 min^{-1}	% inhibition by 40 µg/ml actinomycin C
D 243-2B-R$_1$	$P\varrho^+$	–	24.0	80
D 243-2B-p6	$P\varrho^-$	0 (neutral)	20.5	76
D 243-2B-g	$p_7\varrho^-$	0 (neutral)	27.1	69
D 243-2B-120	$p_7\varrho^-$	51	12.7	73
D 243-2B-106	$p_7\varrho^-$	78	14.0	72
D 243-2B-116	$p_7\varrho^-$	95	12.5	60
Z 5	$p_7\varrho^+$	–	14.1	61
S 19	p ϱ^+	–	26.8	67
S 9×9	p ϱ^+	–	17.2	63

Average values from 3 to 5 experiments are presented. [a] As defined by EPHRUSSI and GRANDCHAMP[16].

[1] J. C. MOUNOLOU, H. JAKOB and P. P. SLONIMSKI, Biochem. biophys. Res. Commun. *24*, 218 (1966).
[2] F. SHERMAN and P. P. SLONIMSKI, Biochim. biophys. Acta *90*, 1 (1964).
[3] Š. KUŽELA and E. GREČNÁ, Experientia *25*, 776 (1969).
[4] Š. KUŽELA, P. ŠMIGÁŇ and L. KOVÁČ, Experientia *25*, 1042 (1969).
[5] J. ŠUBÍK, Š. KUŽELA, J. KOLAROV, L. KOVÁČ and T. M. LACHOWICZ, Biochim. biophys. Acta, in press.
[6] E. WINTERSBERGER and H. TUPPY, Biochem. Z. *341*, 399 (1965).
[7] E. WINTERSBERGER, in *Regulation of Metabolic Processes in Mitochondria* (Ed. J. M. TAGER, S. PAPA, E. QUAGLIARIELLO and E. C. SLATER; Elsevier, Amsterdam 1966), p. 439.
[8] D. J. SOUTH and H. R. MAHLER, Nature *218*, 1226 (1968).
[9] L. KOVÁČ and K. WEISSOVÁ, Biochim. biophys. Acta *153*, 55 (1968).
[10] L. KOVÁČ, H. BEDNÁROVÁ and M. GREKSÁK, Biochim. biophys. Acta *153*, 32 (1968).
[11] S. LEON and H. R. MAHLER, Arch. Biochem. Biophys. *126*, 305 (1968).
[12] K. BURTON, Biochem. J. *62*, 315 (1964).
[13] G. SCHATZ, H. TUPPY and J. KLIMA, Z. Naturforsch. *18b*, 145 (1963).
[14] H. FUKUHARA, M. FAURÈS and C. GENIN, Molec. gen. Genetics *104*, 264 (1969).
[15] E. WINTERSBERGER, Z. physiol. Chem. *348*, 1701 (1967).
[16] B. EPHRUSSI and S. GRANDCHAMP, Heredity *20*, 1 (1965).

The ability of the mitochondria isolated from nuclear (p), cytoplasmic (ϱ^-) and double ($p\varrho^-$) respiratory-deficient yeast mutants to incorporate ^{3}H-UTP into trichloroacetic acid-insoluble fraction shows that, at least in the strains tested, mitochondrial protein synthesis is blocked at the level of translation[17].

Zusammenfassung. Aus verschiedenen cytoplasmatischen (ϱ^-) und nuklearen (p) atmungsdefekten Hefemutanten isolierte Mitochondrien weisen eine Aktinomycin-empfindliche Inkorporation von ^{3}H-UTP in die TCS-unlösliche Fraktion auf. Die Absenz der mitochondrialen Proteosynthese dieser Mutanten könnte daher auf einen Hemmer im Translationsmechanismus zurückgeführt werden.

Š. Kužela and H. Fečíková

Department of Biochemistry, Komensky University, Bratislava (Czechoslovakia), 9 March 1970.

[17] The authors wish to thank Dr. L. Kováč for valuable discussions.

Different ATPase Systems in Glycophytic and Halophytic Plant Species

In a recent review on the halophyte problem, Jennings[1] concluded that with regard to their response to sodium, halophytes differ from mesophytes only quantitatively and not qualitatively. Increased light, aridity and high sodium concentrations had a similar effect on plant cells of various ecological characteristics, namely a change in the ATP metabolism.

In general, there is little information on the influence of saline media on enzymatic activity in animal[2,3] and plant tissues[4,5]. Part of the information regarding ATPase activity concerns its role in ion transport[6–9]. However, relatively little is known of the presence of various ATPase systems in plants of different ecological groups[10], and an investigation into this problem seemed worthwhile.

Two glycophytic species, i.e. bean (*Phaseolus vulgaris* L. c.v. *Brittle wax*) and corn (*Zea Mays* L. c.v. *White horse tooth*), and 2 halophytic species, i.e. *Suaeda monoica* Forsk. and *Atriplex halimus* L. were used for the following investigation. Plants were grown in an aerated Hoagland's nutrient solution for 10 days (bean and corn) and 35 days (*Suaeda* and *Atriplex*) respectively. By that time, the plants were at a more or less equal phase of growth. Sodium chloride was then added to the growth media of half of the plants, so as to give a final concentration of 30 mM. After 3 days the roots were harvested. Tissue fractionation and ATPase activity determinations were performed according to Gruener and Neumann and Neumann and Gruener[11,12]. Homogenization of the roots was performed by grinding the tissue in a cold mortar in a medium containing *Tris* 0.04 M-sucrose 0.5 M. The homogenate was filtered through a sheet of gauze and centrifuged for 5 min at $200 \times g$ to remove unground cells and wall debris. The mitochondria were separated by centrifugation for 20 min at $20{,}000 \times g$ and the microsomes by centrifugation for 60 min at $120{,}000 \times g$. The fractions were dialyzed for 30 h against *Tris* 3×10^{-3} M-EDTA $5 \times 10^{-4} M$, ptt. 7.7, at 5 °C. The solution was

[1] D. H. Jennings, New Phytol. *67*, 899 (1968).
[2] J. M. Augenfeld, Life Sci. *8*, 973 (1969).
[3] M. Thuet, P. Thuet and J. Philippot, C. r. Acad. Sci., Paris *269*, 233 (1969).
[4] M. V. Zhukovskaya, G. P. Matukhin, Sbornik *Rol mineralnikh elementov v obmene veshchestv i produktivmost' rastenii* Izol. Naukay Moscow 1964), p. 332.
[5] E. Porath and A. Poljakoff-Mayber, Pl. Cell Physiol. *9*, 195 (1968).
[6] J. C. Skou, Physiol. Rev. *45*, 596 (1965).
[7] S. L. Bonting and L. L. Caravaggio, Biochim. biophys. Acta *112*, 519 (1966).
[8] M. R. Atkinson and G. M. Polya, Aust. J. biol. Sci. *20*, 1069 (1967).
[9] J. Fischer and T. K. Hodges, Pl. Physiol. *44*, 385 (1969).
[10] C. T. Horovitz and Y. Waisel, Proc. 18th Internatl. Hort. Congress Tel Aviv (1970), p. 89.
[11] N. Gruener and J. Neumann, Physiologia Pl. *19*, 678 (1966).
[12] J. Neumann and N. Gruener, Israel J. Chem. *5*, 107 (1967).

The effect of NaCl in the growth media and in the reaction mixture on the ATPase activity of various plants

Plant species	Reaction mixture		Growth media				
			— NaCl (A)		+ NaCl (B)		
				% from a		% from a	% from A
Phaseolus vulgaris	— NaCl	a	4.78		7.00		146
	+ NaCl	b	5.06	106	8.04	115	159
Zea Mays	— NaCl	a	2.67		4.85		182
	+ NaCl	b	5.42	203	11.30	233	208
Suaeda monoica	— NaCl	a	4.04		1.87		46
	+ NaCl	b	2.53	63	1.20	64	47
Atriplex Halimus	— NaCl	a	7.02		4.33		62
	+ NaCl	b	4.76	68	2.60	60	55

Data denote μmoles Pi/30 min/mg protein.