3 H. KLECZKOWSKA, A. WIATEROWA AND G. BAGDASARIAN, *Acta Microbiol. Polonica*, 14 (1965) 117.
4 D. J. KROGSTAD AND J. L. HOWLAND, *Biochim. Biophys. Acta*, 118 (1966) 189.
5 C. H. FISKE AND Y. SUBBAROW, *J. Biol. Chem.*, 66 (1925) 375.
6 B. L. HORECKER AND W. A. WOOD, in S. P. COLOWICK AND N. O. KAPLAN, *Methods in Enzymology*, Vol. III, Academic Press, New York, 1957, p. 152.
7 J. L. HOWLAND, *Biochim. Biophys. Acta*, 77 (1963) 419.
8 A. F. BRODIE AND J. ADELSON, *Science*, 149 (1965) 265.

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Binding of nucleotides to "structural protein" of wild-type and respiration-deficient yeast mitochondria

One distinguishing feature of "structural protein" isolated from beef heart mitochondria is its capacity to combine with nucleotides such as ATP, ADP and NADH (ref. 1) and a number of other anionic compounds[2]. The binding of nucleotides to "structural protein" was recently shown to be inhibited by atractyloside[3] which is known to block oxidative phosphorylation in intact mitochondria[4,5]. We wish to report that studies on the binding of nucleotides, and its inhibition by atractyloside, provide a valuable means of comparing and identifying mitochondrial "structural proteins" from wild-type bakers' yeast and from its *petite* mutant.

The *Saccharomyces cerevisiae* strain D-273-10B (αP_ρ^+ haploid) and the cytoplasmic *petite* mutant D-273-10B-1 (αPI^- haploid) derived from the wild-type strain by treatment with acriflavin, were grown for 24 h at 26° in the medium described by EPHRUSSI AND SLONIMSKI[6] which was modified to contain 0.3 % yeast extract and 0.8 % glucose. The cells were broken by shaking with glass beads in a MERKENSCHLAGER disintegrator[7] and the mitochondria isolated by differential sedimentation followed by isopycnic centrifugation in a linear sucrose gradient[8]. "Structural protein" was prepared according to the procedure of RICHARDSON, HULTIN AND FLEISCHER[9]. Electrophoresis in polyacrylamide gels revealed that the "structural protein" consisted of several components, one of which was missing in preparations from the non-respiring *petite* mitochondria[10]. Immunological studies also indicated that mitochondrial "structural protein" from the *petite* mutant lacked a component present in a corresponding preparation from the wild-type yeast[10]. Mitochondrial "structural protein" (1.2 mg) suspended in Tris acetate buffer, pH 7.5, was incubated with 10 mμmoles of [^{3}H]ATP. This amount of ATP was much smaller than that required for saturating the binding capacity of "structural protein". After 10 min at 25° the "structural protein" was sedimented by low-speed centrifugation. The amount of [^{3}H]ATP bound to the protein was estimated from the difference between total activity and radioactivity remaining in the supernatant solution (Table I). About 70 % of the added [^{3}H]ATP were bound to the wild-type "structural protein". Similar results were obtained when [^{3}H]ATP was replaced by [^{3}H]TTP. ATP binding was not significantly diminished in the presence of equimolar amounts of ADP or NADH. Even a 10-fold excess of ADP reduced the amount of bound [^{3}H]ATP by not more than one-half. In contrast, an equimolar amount of atractyloside strongly inhibited

the binding of ATP. Alternatively, when the preformed complex between "structural protein" and [^{3}H]ATP was incubated with atractyloside for 10 min at 25°, the protein-bound ATP was almost completely discharged. "Structural protein" isolated from the mutant mitochondria bound much less ATP than the protein from the wild type.

TABLE I

BINDING OF [^{3}H]ATP TO "STRUCTURAL PROTEINS" FROM DIFFERENT CELL FRACTIONS AND ITS INHIBITION BY ATRACTYLOSIDE

The cell fractions were prepared at 0–4°. The isolation of "structural protein" (0–4°) and the determination of nucleotide binding were described in the text. The incubation medium contained 1.2 mg protein, 10 mμmoles [^{3}H]ATP (specific activity: 40 mC/mmole), 1.2 ml Tris acetate (50 mM, pH 7.5) and, where indicated, 10 mμmoles atractyloside.

	mμmoles of [^{3}H]ATP bound to protein		*Number of experiments*
	in the absence of atractyloside	*in the presence of atractyloside*	
Mitochondrial "structural protein"			
from wild-type yeast	7.2 ± 0.9	2.1 ± 1.4	8
from mutant yeast	1.5 ± 0.6	0.7 ± 0.3	5
Microsomal "structural protein"			
from wild-type yeast	9.5 ± 0.3	9.5 ± 0.3	2
from mutant yeast	9.4 ± 0.2	9.4 ± 0.2	2
105000 × *g* supernatant "structural protein"			
from wild-type yeast	1.2 ± 0.3	1.1 ± 0.3	2
from mutant yeast	0.2	0.2	2

An obvious assumption would be that the greatly diminished ATP affinity of the mutant protein was due to the fact that it lacked at least one component present in the corresponding wild-type protein[10]. However, *petite* mutant mitochondria which were isolated at room temperature (17–20°) rather than in the cold (0–4°) yielded a "structural protein" whose binding capacity for nucleotides equaled that of the wild-type protein (Table II). Judging from immunological experiments, the composition

TABLE II

BINDING OF [^{3}H]ATP TO "STRUCTURAL PROTEIN" FROM YEAST MITOCHONDRIA ISOLATED AT 0–4° AND AT 17–20°

The incubation conditions were the same as those given in Table I.

	mμmoles [^{3}H]ATP bound to protein	*Number of experiments*
"Structural protein" from wild-type yeast		
mitochondria isolated at 0–4°	7.2 ± 0.9	8
mitochondria isolated at 17–20°	7.6 ± 0.6	3
"Structural protein" from *petite* mutant yeast		
mitochondria isolated at 0–4°	1.5 ± 0.5	5
mitochondria isolated at 17–20°	6.7 ± 0.8	3

of the "structural protein" did not change with the temperature at which the mutant mitochondria were isolated[10]. One might thus speculate that the *petite* mutation causes the loss of a component present in the "structural protein" and thereby induces cold-lability of the atractyloside-sensitive nucleotide binding. This finding is reminiscent of results obtained by SCHATZ[11] which indicate that ATPase (F_1) associated with the mutant yeast mitochondria is cold-labile, whereas the enzyme bound to mitochondria of the wild-type strain is cold-stable.

Recently, WOODWARD AND MUNKRES[12] reported that the *poly* mutation in *Neurospora crassa* affected not only the "structural protein" of mitochondria, but also "structural proteins" similarly prepared from the nuclear, the microsomal and the microsomal-supernatant ("soluble") fractions. The authors suggested that "structural proteins" isolated from different cell fractions were identical. In the present experiments with yeast, however, the occurrence of a "structural protein" common to mitochondria and the other cell fractions could not be confirmed. "Structural protein" was prepared from a post-mitochondrial "microsomal fraction" as described above for the mitochondrial protein. A fraction comparable to "structural protein" was prepared from the 105000 $\times$ *g* supernatant by precipitation with 12 % saturated ammonium sulfate and delipidation with 90 % acetone. "Structural protein" from microsomes, like that from mitochondria, exhibited a strong affinity for ATP. However, ATP binding by the microsomal protein was insensitive to atractyloside (Table I). The insoluble protein fraction isolated from the 105000 $\times$ *g* supernatant differed from both mitochondrial and microsomal "structural protein" by its failure to bind any significant amount of ATP.

The present results thus disclose yet another striking difference between mitochondrial "structural proteins" isolated from wild-type and from *petite* yeast cells. In addition, they strongly argue against the claim[12] that "structural proteins" isolated from different cell fractions are identical.

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1 H. O. HULTIN AND S. H. RICHARDSON, *Arch. Biochem. Biophys.*, 105 (1964) 288.
2 F. PALMIERI AND M. KLINGENBERG, *Biochim. Biophys. Acta*, 131 (1967) 582.
3 V. MORET, M. LORINI, A. FOTIA AND N. SILIPRANDI, *Biochim. Biophys. Acta*, 124 (1966) 433.
4 A. BRUNI AND A. R. CONTESSA, *Nature*, 191 (1961) 818.
5 P. V. VIGNAIS, P. M. VIGNAIS AND E. STANISLAV, *Biochim. Biophys. Acta*, 51 (1961) 394.
6 B. EPHRUSSI AND P. P. SLONIMSKI, *Biochim. Biophys. Acta*, 6 (1950) 256.
7 M. MERKENSCHLAGER, K. SCHLOSSMANN AND W. KURZ, *Biochem. Z.*, 329 (1957) 332.
8 G. SCHATZ, E. HASLBRUNNER AND H. TUPPY, *Biochem. Biophys. Res. Commun.*, 15 (1964) 127.
9 S. H. RICHARDSON, H. O. HULTIN AND S. FLEISCHER, *Arch. Biochem. Biophys.*, 105 (1964) 154.
10 H. TUPPY, P. SWETLY AND I. WOLF, in preparation.
11 G. SCHATZ, *J. Biol. Chem.*, submitted for publication.
12 D. WOODWARD AND K. MUNKRES, *Proc. Rutgers Symp.*, (1966).

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