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8. We thank the members of the Laboratory of Physiology, Ocean Research Institute, University of Tokyo, for teaching us embryological techniques; Dr. A. Kanemitsu of the University of Tokyo for preparing Fig. 1; Prof. S. Hagiwara and Dr. A. D. Grinnell for their comments on this manuscript; and the marine biological laboratory at Asamushi of Tohoku University, the Marine Laboratory of Fishery at Onagawa of Tohoku University, and Otaru City Aquarium and Wakkanai City Aquarium in Hokkaido for collecting materials. Supported by a grant from the Japan Educational Ministry to K.T.

7 August 1970; revised 20 October 1970 ■

Genetic Restriction of Energy Conservation in Schizophyllum

Abstract. *A genetically determined malfunction of energy conservation reduces assimilation of substrate to half or less its normal level while leaving oxidative processes apparently unchanged. This metabolic defect, which differs in many ways from any previously described, is related to the function of the* B *incompatibility factor and occurs both in the common* A *heterokaryon* (A = B ≠)*—in which two compatible, wild* B *factors interact—and in mutant-*B *homokaryons.*

A malfunction of energy metabolism that differs from any previously known occurs as a normal feature in the tetrapolar basidiomycete *Schizophyllum commune*. Previously reported cases of genetically altered respiratory processes are of two general types. In *petite* mutants of yeast and *mi* or *poky* mutants of *Neurospora*, critical oxidative processes are absent or severely reduced (*1*). Several mutations in yeasts that affect later stages in the respiratory system have been found in a search for mutants that have intact oxidative systems uncoupled from adenosine triphosphate (ATP) formation (*2*). The phenomenon reported here more closely resembles the latter type of defect but differs in several significant ways from any of the cases now known.

Perhaps a closer parallel is found in the high rate of respiration leading to thermogenesis and little generation of ATP in brown adipose tissue of certain mammals (*3*).

The metabolic malfunction in *Schizophyllum* is associated with a specific phenotype that results from either of two distinct genotypes. Sexual development follows from the mating of two homokaryons that are heteroallelic for both of two incompatibility factors, the *A* factor and the *B* factor. A mating between two homokaryons that differ at only one factor results in the operation of only part of the morphogenetic sequence. Matings where the *A* factor is homoallelic and the *B* factors heteroallelic ($A = B \neq$) yield heterokaryons with greatly reduced growth and altered hyphal morphology with gnarled, irregularly branched hyphae and frequent protoplasmic extrusions (*4, 5*). This heterokaryon is very closely mimicked for every aspect studied by a homokaryon carrying a primary mutation in the *B* factor. The phenotypic similarities of the two types of mycelia include gross and microscopic morphology, nuclear distribution, sexual interactions, ultrastructure, and enhanced glucanase activity (*6, 7*). Identical developmental controls seem to be released by two compatible interacting *B* factors in the heterokaryon and by the single mutant-*B* factor in the homokaryon (*7–9*).

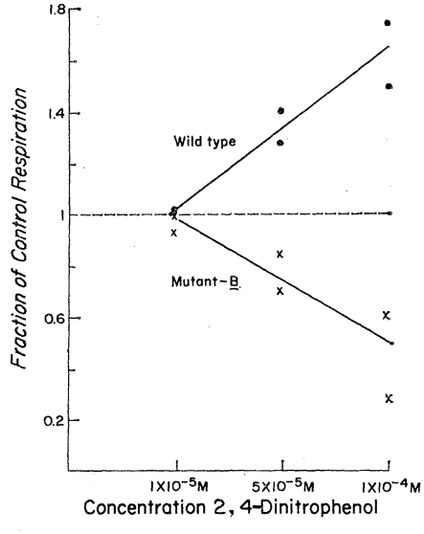

Fig. 1. Effect of DNP on respiration of mutant-*B* and wild-type homokaryons. Strains were grown in liquid for 2 to 3 days and washed twice; respiration was measured manometrically. Each respirometer flask contained 3 ml of mycelial suspension, 0.1*M* phosphate buffer at *p*H 6, an appropriate concentration of DNP in the main chamber, and 10 percent KOH in the central well.

Our study has been made primarily with the mutant-*B* homokaryon—a homogeneous system in contrast to the $A = B \neq$ heterokaryon, which contains homokaryotic as well as heterokaryotic elements (*5, 8*).

Studies on the *B* mutant at 30°C in shaking-liquid cultures in minimal medium containing 2 percent glucose have shown values for growth rate, total growth capability, and ratio of dry weight formed to glucose used reduced to half or less compared to the corresponding values for a wild-type strain that is isogenic to the mutant except for the *B* factor. Residual glucose in the medium was measured by an enzymatic assay (Glucostat Special, Worthington). Growth was measured by increase in dry weight, determined by filtering the mycelium on tared filter paper and drying at 105°C for 24 hours. These results parallel the preliminary findings for the $A = B \neq$ heterokaryon (*5*). Enriched media such as yeast extract, peptone, and malt extract do not restore the mutant to normal growth.

Respiration of these obligate aerobes (there was no detectable growth after 9 days in a tank flushed with nitrogen and maintained under positive pressure) was measured manometrically (*10*) at 30°C on a dry weight basis. Mycelia were grown in a liquid, minimal, glucose medium for 2 to 3 days; the cultures were incubated on a rotary shaker, and the harvested cells were washed twice by centrifugation. The mycelial pellets were then suspended in the above growth medium, and a portion (3 ml) was pipetted into each respirometer flask. Respiration was measured for 80 minutes. The endogenous rates of the wild-type strain correspond to those found previously (*11*). The mutant *B* showed an accelerated respiration rate (Table 1).

To determine whether, in the mutant, the inefficient use of glucose and the accelerated respiration rate were linked to a more extensive oxidation of glucose, we grew and prepared mycelia in two ways: (i) as above for the determination of respiratory rates and (ii) colonies were washed after 3 to 4 days' growth from cellophane membrane on minimal medium. Measurements were made manometrically (at 30°C) of CO_2 production and O_2 consumption

Table 1. Rates of respiration of wild-type and mutant-*B* homokaryons. The oxygen uptake is based on dry weight; the rate is expressed as microliters per milligram per hour.

Strain	Uptake of O_2 per hour	
	Endogenous	Exogenous with 2 percent glucose
Wild type	7 ± 0.22	14.2 ± 0.25
Mutant *B*	14.6 ± 0.047	29.8 ± 1.87

per unit of glucose used in a defined growth medium with glucose as the sole carbon source. For each strain, a number of respirometers were run simultaneously. Glucose was tipped into one half of them to a final concentration of 1 mg/ml, and water was tipped into the other half. The endogenous respiration rates were subtracted from the exogenous rates; when the two rates became equal, it was assumed that all the glucose was utilized, and the experiment was terminated (*10*). The respirometer flasks were sterilized to permit measurements under axenic conditions. The *B* mutant and the $A = B \neq$ heterokaryon oxidized more of the glucose molecule than did the wild type by a factor of about 2 (Table 2). This result, together with the reduced growth per unit of glucose consumed by the mutant and the heterokaryon vis-à-vis the wild-type homokaryon, suggests that, although the oxidative capacity is retained, the mutant and the heterokaryon lack the ability to conserve normal amounts of energy from substrate oxidation. The slow growth of the mutant and the heterokaryon may thus be due to reduced energy conservation resulting in less assimilation of glucose into cell material.

The wild-type values agree with manometric determinations on *Escherichia coli* (*12*), with ^{14}C radiorespirometric data on *Salmonella typhimurium* (*13*), and with calorimetric and chemical data on *Saccharomyces cerevisiae* (*14*), all of which oxidize about 25 percent of the exogenous glucose.

We have found anomalous behavior of the mutant toward 2,4-dinitrophenol (DNP), the classic uncoupler of oxidative phosphorylation (*15*). In manometric determinations at 30°C, DNP in concentrations from 10^{-5} to $10^{-4}M$ in phosphate buffer (0.1*M*, *p*H 6) *decreased* the rate of the mutant's respiration in contrast to that of the wild type, which had an expected increase in respiration (Fig. 1). At $10^{-4}M$ DNP with a starting *p*H of 6, the growth of the mutant was unaffected, whereas the growth of the wild type was reduced to one-half normal. It has been shown that DNP can prevent assimilation of cell material without reducing oxidative capacity (*16*), as is the case in the mutant without inhibitor. One possible mechanism for this behavior by the mutant would be the inefficient coupling of oxidative phosphorylation. This could be responsible for the postulated reduced ability of the mutant to conserve energy from oxidation of substrate.

2-Deoxyglucose at $1.9 \times 10^{-2}M$, an inhibitor of glycolysis and cell wall polysaccharide synthesis (*17*), completely inhibits the mutant's growth but allows significant although reduced growth of the wild type. In view of this finding and the insensitivity of the growth of the mutant to $10^{-4}M$ DNP, we conclude that the mutant probably depends heavily on the energy of glycolysis.

The mutants affecting oxidative phosphorylation known in yeast differ in many ways from the mutant-*B* homokaryon described here. The mutant op_1 has an inefficient oxidative metabolism, but its respiration is still stimulated by DNP (*3*, *18*), an observation that led to the conclusion for op_1 that "the first step in the energy conservation mechanism, the coupling process, is preserved and that the subsequent reactions are affected by the mutation" (*3*). In another study of 35 mutant yeast strains with inefficient oxidative metabolism, three were found in which DNP appeared not to stimulate respiration (*19*). Two of these, however, had respiratory rates much lower than the wild type and grew less than half as much on glucose as the wild type. The third yielded about 90 percent as much growth as the wild type on glucose and had respiratory rates lower than normal at 24 hours and higher at 48 hours (*19*). It thus appears that the mutant-*B* homokaryon of *S. commune* is quite unique physiologically and may represent the first characterized mutant demonstrating a partial uncoupling of a normally functioning oxidative system from the process of energy conservation. The data in Table 2 [see (*5*)] indicate a point-by-point metabolic correspondence of the mutant-*B* homokaryon and the $A = B \neq$ heterokaryon. Full confirmation of the defect in the $A = B \neq$ heterokaryon will constitute the first instance of a severe metabolic defect as the result of the interaction of wild-type genes.

The phenomenon described raises two intriguing questions: What can be the rationale of a serious metabolic malfunction resulting from the interaction of two wild alleles of the *B* factor? How does the added presence of a pair of interacting *A* factors (the only genetic difference between the $A = B \neq$ heterokaryon and the dikaryon) effect a restoration of vegetative vigor in the dikaryon?

Table 2. Stoichiometry of O_2 uptake and CO_2 production per unit of glucose consumed by wild-type and mutant-*B* homokaryons and $A = B \neq$ heterokaryon.

Grown before harvest (hr)	Strain	Molar ratios	
		CO_2/glucose	O_2/glucose
	Liquid-grown mycelia		
68	Wild type	1.3	1.23
54.5	Wild type	1.68	1.32
75	*B* mutant	3.4	2.7
70	*B* mutant	4	3
	Membrane-grown mycelia		
	Wild type 1		1.3
	Wild type 2		1.8
	$A = B \neq$ heterokaryon*		3.5

* Constituted of wild types 1 + 2.

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20. Supported by a predoctoral traineeship to R.M.H.

14 September 1970