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Previous studies have demonstrated that the phenotypic character of the cytoplasmic mutant mi-1 can be transmitted to recipient wild type cell by micro-injection of isolated mi-1 mitochondria. During serial transfers from the injected cell, a phenotypic lag occurs before the mi-1 phenotype appears. The present study is an examination of the developing transfers.

Polarographic studies were conducted with intact cells and isolated mitochondria of critical transfers in the presence of the respiratory inhibitors cyanide and salicyl hydroxamic acid (SHAM). SHAM inhibits cyanide-resistant respiration in higher plants and Neurospora.

Without inhibitors, the respiration rates of early transfers were equal to wild type rates. A gradual increase occurred, after which transfers remained above the wild type level, thus displaying a stable non-reverting characteristic.

For whole cells and isolated mitochondria of wild type and early transfers, inhibition of respiration by cyanide was complete. At Transfer six there was an abrupt resistance to cyanide as is characteristic of mi-1.

In whole cells and isolated mitochondria, SHAM-sensitive respiration develops between Transfers five and eight. Although significantly above wild type levels, this SHAM-sensitivity does not reach the level characteristic of mi-1.

In whole cells and isolated mitochondria, cyanide-resistance and SHAM-sensitivity do not develop simultaneously. The beginning of SHAM-sensitivity is at Transfer five, while cyanide-resistance does not develop until Transfer six. The major difference is the onset: SHAM-sensitivity gradually increases while cyanide-resistance develops in a single transfer. The difference in the development of SHAM-sensitivity and cyanide-resistance suggests that the mechanisms conferring these characteristics are not identical.

EFFECTS OF RESPIRATORY INHIBITORS ON INTERMEDIATE STAGES IN THE DEVELOPMENT OF THE *mi-1* PHENOTYPE IN *NEUROSPORA*

by

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APPROVAL SHEET

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CHAPTER I

INTRODUCTION

Characteristics of Wild Type and mi-1

Neurospora crassa is a fungus in the class Ascomycetes. It is found naturally in tropical or subtropical areas growing on trees and celluosic plant remains. The sexual and vegetative life cycles of Neurospora are well known (Tatum, 1944; Beadle, 1945). Of particular interest is the phenomena of cytoplasmic inheritance displayed by this organism (Mitchell and Mitchell, 1952; Wagner and Mitchell, 1964).

Cytoplasmic or maternal inheritance (mi) is derived from the effect the cytoplasm (contributed by the egg) has on the phenotype of an organism. There is evidence for maternal effects (extrachromosomal inheritance) caused by the DNA of mitochondria or chloroplasts etcetera which are transmitted independently of the nuclear genome. Such is the case for the respiratory deficient mutants of Saccharomyces and Neurospora.

Saccharomyces cerevisiae, another ascomycete, is shown to exhibit maternal inheritance. The vegetative neutral petite mutation is similar to mi-1 of Neurospora in that it grows slowly and has an abnormal cytochrome chain. The cytoplasmic agent (rho) responsible is suggested to be the mitochondrial DNA (Gillham, 1974). The organism and its characteristics are reviewed more thoroughly by Gillham (1974).

The strain mi-1, better known and best described as "poky" because of its slow growth, arose spontaneously in a stock culture of a standard wild type of Neurospora (Mitchell and Mitchell, 1952). In the sexual

cycle when nuclear genes segregate they do so with a resulting 4:4 ratio. All mi-1 progeny result when mi-1, the protoperithecial parent, is crossed to wild type. This is a non-mendalian segregation typical of cytoplasmic inheritance. In the reciprocal cross all progeny are wild type (Mitchell and Mitchell, 1952).

In mi-1 the lesion responsible for its phenotype is not associated with the mitochondrial DNA. Rifkin and Luck (1971) suggest that cytochrome deficiency results from absence of a normal mitochondrial ribosomal RNA species. This suggestion is supported by the observation that as mi-1 ages there is an increase in cytochrome oxidase activity and an increase in the monomeric ribosome. This indicates regulatory gene mutation rather than a structural one as thought to be the case in the petite mitochondrial DNA.

At least nine cytoplasmically inherited mutants originated spontaneously. They are characterized as cytoplasmic mutants because of abnormal cytochromes, maternal inheritance, transmission in heterokaryon test, and infective spreading in a culture (Gillie, 1972). Gillie and later Edwards et al (1973) provided methods for selecting and isolating respiratory mutants which could be cytoplasmic mutants.

In wild type the earliest period of incubation (first six hours) of conidia is the time of intense metabolic activity (Greenawalt, Beck, and Hawley, 1971). Protein and phospholipid synthesis reach their maximum at the same time as the respiration rates. The increase in mitochondrial number in the first six hours does not correspond with the increased respiration rate. The increase in mitochondrial protein synthesis contributes to the increase in respiratory and phosphorylating capacities of

the mitochondria. In young cells the ADP:O and respiratory control ratios are lower than those of older cells with the maximal rate occurring at six hours (Grenawalt et al, 1971).

The wild type growth rate is eight times greater than that of mi-1. This rate continues as long as 144 hours (Mitchell and Mitchell, 1952). The doubling time of wild type is about twice that of mi-1 (f-) (Lambowitz and Slayman, 1971). The expontial growth phase of wild type is 12-14 hours whereas, it is six to 22 hours for mi-1 at 25° C. in liquid medium (Slayman, Rees, Orchard, and Slayman, 1975). Wild type ascospores complete their growth on slants in 3 to 4 days as compared to mi-1 which takes 10 to 12 days (Mitchell and Mitchell, 1952). A supressor f observed for mi-1 increased the growth rate (f +) to that of wild type. However, the cytochrome pattern and respiration rates remained the same as mi-1 (f-)(Lambowitz and Slayman, 1971).

Wild type contains less free fatty acids than mi-1 (Hardesty and Mitchell, 1963). Free fatty acids similar to those in mi-1 made horse heart cytochrome c more sensitive to peroxide oxidation, unreducible by ascorbate, auto-oxidizable, and hypochromic in the Soret absorption bands (Hardesty and Mitchell, 1963b).

Tissieres, Mitchell and Haskins (1953) found that whole cells and cell free extracts from 40 hour mi-1 cultures are insensitive to cyanide and azide. Later, Lambowitz and Slayman (1971) characterized the cyanide insensitive respiration in whole cells of wild type and mi-1 (f- and f +). In exponential growth phase, where oxygen is not rate limiting, wild type demonstrated respiration rates 50 percent lower than exponentially growing mi-1. As mi-1 ages from 12 to 24 hours

respiration remains constant; whereas, wild type in a comparable time declines 25 percent (Lambowitz and Slayman, 1971).

Cyanide, azide (Tissieres et al, 1953; Lambowitz and Slayman, 1971), and antimycin A (Lambowitz and Slayman, 1971) completely inhibited wild type whole cell respiration. Salicyl hydroxamic acid (SHAM), an inhibitor of cyanide insensitive respiration in plants (Schonbaum, Bonner, Storey, and Bahr, 1971), decreased respiration in mi-1 cells about 70 percent. SHAM showed no effect on wild type (Lambowitz and Slayman, 1971).

The mitochondrial light absorption spectrum of mi-1 is dominated by flavoproteins, pyridine nucleotides, and cytochromes which are similar to wild type (Haskins, Tissieres, Mitchell and Mitchell, 1953; Hardesty, 1961; Rifkin and Luck, 1964; Lambowitz, Smith and Slayman, 1972). The pyridine nucleotides in the form of NAD^+ and NADH of mi-1 are present in only one half the amount observed in wild type. Wild type has 50 percent less flavoprotein and cytochrome c, but 70 percent more cytochrome a/a_3 than mi-1. Both mi-1 and wild type have two cytochrome components (Lambowitz et al, 1972a). Lambowitz et al (1972a) revealed a significant amount of cytochrome b and a/a_3 in mi-1 which is in contrast to previously observed value of Haskins et al (1953). This is an interesting result since Edwards and Woodward (1969) found cytochrome c oxidase activity in mutants with cytochrome spectra similar to that of mi-1.

Cytochrome c and c_1 are present in both wild type and mi-1 (Lambowitz et al, 1972). Cytochrome c from both wild type and mi-1 analyzed by Heller and Smith (1966) is identical. In mi-1 only half of the cytochrome c present is bound to the mitchondrial membrane