Fungal Genetics Reports

Volume 13 Article 2

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Recommended Citation

Griffiths, A. J., H. Bertrand, and T.H. Pittenger (1968) "Cytochrome spectra of cytoplasmic mutants," *Fungal Genetics Reports*: Vol. 13, Article 2. https://doi.org/10.4148/1941-4765.1935

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Abstract

Cytochrome spectra of cytoplasmic mutants

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Cytochmme spectra of cytoplasmic mutants in Neurospora

Definitive studies on the absorption spectra of the Neurospora cytoplasmic mutants [poky] and [mi-3] were originally performed by Mitchell et al. (1953 Proc. Natl. Acad. Sci. U. S. 39: 606) and Tissieres and Mitchell (1954 J. Biol. Chem. 208: 241). Their studies were done with a hand spectroscope on mycelial pads and crude mitochondrial suspensions. The present work essentially repeats their experiments, but derives the cytochmme spectra spectrophotometrically from disrupted mitochondrial preparations. Other maternally-inherited mutants ore also examined.

Mitochondrio were prepared by a method similar to that used by Luck (1965 J. Cell Biol. 24: 445). Mycelium was grown in liquid shake cultures at 30°C and harvested in the exponential growth phase. After grinding with sand in 0.01 M Tris buffer containing 0.001 M EDTA (adjusted to pH 7.3) and 0.44 M sucrose, cell debris was removed by two 10 minute centrifugations at 1000 x g. Mitochondria were spun down in a 30 minute centrifugation at 20,000 x g and washed once in the buffered sucrose. The resulting crude mitochondrial pellets were disrupted by sonication and the solutions cleared by adding sodium deoxycholate to a concentration of 2%. Spectra were read in a Cary 16 spectrophotometer, a few crystals of sodium dithionite being added to the sample cuvette to reduce the cytochromes. All the spectm were read from solutions containing IO-20 mg/ml of protein, estimated by the Folin test.

It has been found that the cytoplasmic mutants tested fall into two groups on the basis of their spectra. The first group, consisting of [poky] (3627-Z) (FGSC#384), suppressed [poky] ([polpoky] 3627-3 and 3627-4) (FGSC#'s 386 and 385)), [SG-3] (no isolation #, FGSC#1452), a UV-induced stopper strain ([stp] 30a 4, FGSC#1573 McDougall and Pittenger 1966 Genetics 54: 551), and two stopper strains spontaneously arisen in separate continuous growth tubes ([stp-A] A40-4, and [stp-B] 17-2a-1, Bertmnd and Pittenger 1968, in preparation). All of these strains show identical mutant spectra of the type shown in Figure 1. The notable features are an absence of cytochromes a (610 mμ) and b (560 mμ), and a very marked a-cytochrome c peak (550 mμ). The published data of Diacumakos et al. (1965 J. Cell Biol. 26: 427) reveal that [abn-1] also belongs to this group. A typical wild type spectrum is shown in Figure 2. r & o r [mi-1] FGSC#343 exhibited a wild type spectrum.

The second group consists of the [mi] strains, [mi-2] to [mi-8] (mi-2R1 to mi-7RI and mi-8R6) (FGSC#'s 1233, 383, 1234, 1235, 1236, 1237, 1238), and a typical spectrum is shown in Figure 3. Cytochrome a is again absent, cytochrome b is present in wild type amounts, and cytochmme c is again in excess. In the work of Mitchell et al. a strong band was obsewed at 590 mμ in [mi-31, and was labelled cytochmme a_1; this has never been observed in our experiments. The [mi] strains [mi-2] to [mi-8] are in fact probably replicates of the same mutant (M. B. Mitchell, personal communication).

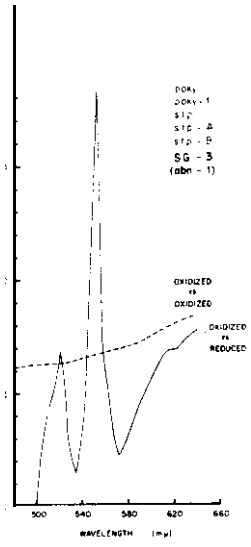

FIGURE 1

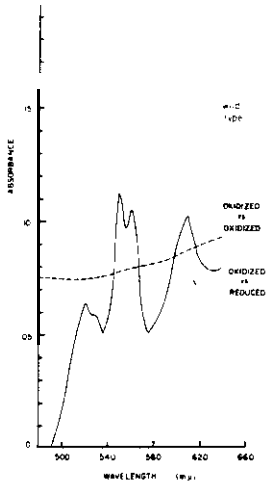

FIGURE 2

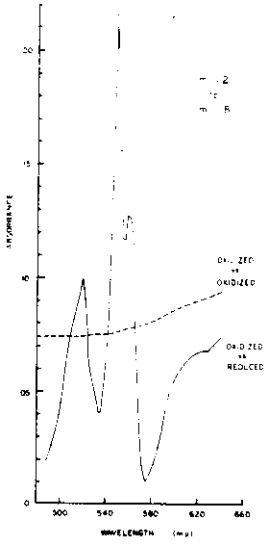

FIGURE 3

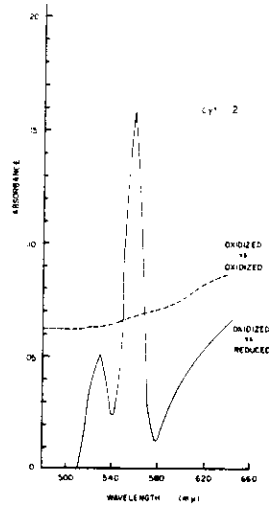

FIGURE 4

On occasion (mi-31 A (FGSC#383) has shown a spectrum closer to that of wild type. The cause of this apparent reversion is not known, but it has also been observed by other workers (Grindle and Woodward 1967 Neurospora Newsl. 12: 9).

The two nuclear genes known to affect cytochrome content in Neurospora hove also been examined cyt-2 (C117) (FGSC #339) is shown in Figure 4, and is similar to the spectrum obtained for this strain by Mitchell et al., in t that cytochromes a and c ore both absent, but differs in that no cytochrome e is detected at 553 mμ. cyt-1 (C115) (FGSC#355) shows on essentially wild type spectrum. Tissieres and Mitchell (1954) have, however, indicated that C115 is particularly prone to suppression, so it must be concluded that this is the case in the culture tested. cyt-1 (C115) (FGSC#1217) was not tested.

The ß-peaks of cytochromes c (520 mμ) and b (530 mμ) are seen to vary in accordance with the α-peaks. From the curves it is possible to calculate the absolute amounts of cytochrome present. However, it is apparent that the relative amounts ore more useful in diagnosing mutant types. The fact that the above spectra ore in the main port very similar to the mycelial spectra produced by Mitchell et al. is indicative that the whole-cell cytochrome content reflects, to a large degree, the mitochondrially bound complement, which in turn is presumably dependent on the basic genetic lesion responsible for the maternal inheritonce of the metabolic defects. The gene f does not suppress [mi-3], and the cytoplasmic mutants in the first group described above were induced in a variety of nuclear backgrounds. Thus it seems reasonably certain that the groups represented by [poky] and [mi-3] reflect truly different types of genetic lesions, in two regions of either one or two mitochondrial 'genes', (concerned, perhaps, with structural protein) and ore not nuclear modifications of each other. - - - Division of Biology, Kansas State University, Manhattan, Kansas 66502.