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Lecithin Requirement for the Sporulation Process in *Neurospora crassa*

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Reversible inhibition of conidiogenesis occurred when lecithin was depleted from *Neurospora* membranes by choline starvation.

The process of development of eucaryotic cells is often associated with the synthesis of new lecithin-containing membranes. *Neurospora crassa* is a particularly interesting eucaryotic microorganism because it exhibits both differentiation and aging processes (7). In addition, there are membrane mutants of this microorganism that are impaired in lecithin synthesis in the absence of choline (1). The largest decrease in phosphatidyl choline/phosphatidyl ethanolamine ratio, under conditions of choline starvation, has been detected in the *chol-1* mutant (3). In this work, I used the choline starvation process to detect the stage of *chol-1* development that is most sensitive to lecithin depletion from *Neurospora* membranes. Growth conditions in minimal medium (without choline) and supplemented medium (with 50 μg of choline per ml) have been described previously (4). Sporulation was estimated after spores were harvested with distilled water. The optical density of the water solution was compared with the microscopic count and with the result of plating the spores on L-sorbose medium (2). One million spores per milliliter of water solution corresponded to an optical density of 0.1 at 560 nm (Bausch & Lomb Spectronic 20 colorimeter), whereas the dry weight of 10^6 spores was approximately 0.2 mg.

Growth experiments in the absence of choline were performed to check (i) whether the growth process occurs, (ii) for characteristics of the growth process, and (iii) for reversibility of the growth inhibition.

The germination of *chol-1* spores proceeds at the same growth rate in both minimal and supplemented medium, i.e., with a doubling time of about 2.5 h. After two or three mass doublings, the growth on minimal medium starts to slow down. The total number of mass doublings, after 3 days of growth in minimal medium, does not exceed five or six, with the final growth yield being proportional to the number of spores inoculated. If the inoculum of spores is large enough, it can produce any desired yield of choline-starved mycelium. Addition of choline at any time increases the growth yield to the values characteristic of growth on supplemented medium.

In advanced growth experiments, I regularly observed the inability of choline-starved mycelium to attach conidia on the glass surface of the flask. This observation suggested that starved mycelium might not be able to undergo the metabolic shift associated with the process of conidiation. Conidiation can be synchronized by exposing mycelium to air in the absence of a carbon source (5). In the next experiment, mycelium was grown for 4 days in minimal or supplemented medium, harvested by vacuum filtration, washed, streaked on an agar plate containing only 0.1 M phosphate buffer (pH 6.1), and incubated at 30°C. Choline-starved mycelium remained inactive, whereas supplemented mycelium underwent a vigorous process of conidiophore production; conidiophores spread over the surface of the agar plate after 20 h of incubation. Addition of 1 mg of choline chloride to the phosphate plate with choline-starved mycelium induced synchronous production of conidiophores in a clearly visible cyclic growth pattern (after 20 h of incubation, with time zero taken as the time of choline addition).

In the last set of experiments, L-sorbose agar medium was used to (i) eliminate the role of choline as the growth-limiting factor and to see whether choline could still induce conidiogenesis and (ii) check for viability of *chol-1* spores in the absence of choline.

L-Sorbose medium is known to decrease the growth rate of *N. crassa* to such a degree that colonies are formed, each of which develops from an individual spore (2). *chol-1* spores plated on L-sorbose medium develop colonies

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whether choline is present or not. No difference in viability or colony size can be detected during the first 4 days of incubation at 28°C. On day 5, sporulation is detected only for colonies on choline-supplemented plates. For the next 10 days, sporulation is absent on the plates lacking choline. However, if choline is added to such plates (1 mg), sporulation develops after 2 days.

Choline starvation in the *chol-1* mutant results in depletion of lecithin from *chol-1* membranes—from roughly 40% among other phospholipids to only trace amounts, (D. Juretić, Ph.D. dissertation, Pennsylvania State University, University Park, 1976).

According to the results described here, lecithin is required for conidiogenesis. This requirement is not apparent in wild-type *N. crassa*, which can use phosphatidyl ethanolamine for lecithin synthesis. A number of Krebs cycle enzymes, respiratory enzymes, and glyoxylate cycle enzymes have been shown to be indispensable for the *Neurospora* differentiation process (6). Some respiratory-deficient mutants are also aconidial, whereas aconidial mutants are generally unable to shift their fermentation-oxidation balance toward the oxidative side (6). It has been shown recently (4) that the respiratory characteristics of choline-starved mycelium resemble those of respiratory-deficient mutants. Respiratory chain enzymes located in the inner mitochondrial membrane merit further examination with respect to their requirement for lecithin and their involvement in the *Neurospora* differentiation process.

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