

## Elucidation of "inositol-less death"

P. Matile

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### Abstract

Elucidation of "inositol-less death"

Matile, P. Elucidation of "inositol-less death."

The phenomenon of "inositol-less death" in *Neurospora* is the basis of an efficient and widely used method for the isolation

of heterotrophic mutants (Lester and Gross 1959 *Scions*. 129: 572). Abnormal growth (Beadle 1944 *J. Biol. Chem.* 156: 683) and decline of viability (Stevens and Mylroie 1953 *Nature* 171: 179) of suboptimally cultured inositol-less strains has been explained by an unbalance between the synthesis of inositol phospholipid (a structural constituent of cytoplasmic membranes) and other cellular constituents (Shatkin and Tatum 1961 *Amer. J. Botany* 48: 760). Still, the peculiarity of inositol-less in contrast to other heterotrophic mutants has remained mysterious.

An investigation of the mechanism of the utilization of exogenous proteins in *Neurospora* has led to the detection of a constitutive cytoplasmic particle which contains the proteolytic enzymes to be secreted into protein-containing growth media (Motile 1965 *Z. Zellforsch.* 65: 884). These secretory granules have been termed protease particles (Motile 1964 *Naturwissenschaften* 51: 489); they represent small spheres (diameters from 0.15 to 0.3  $\mu$ ) surrounded by a single membrane (Motile et al. 1965 *Z. Zellforsch.* in press). Incorporation of either choline-C<sup>14</sup> or inositol-C<sup>14</sup> into respective heterotrophic strains followed by cell fractionation (density gradient centrifugation) and analysis of the lipids has shown that the lipid composition of the membranes of protease particles is significantly different from that of other cytoplasmic membranes: they are relatively poor in lecithin and rich in inositol phospholipid.

If an inositol-less strain is cultured at a high level of exogenous inositol (50  $\mu$ g/ml), the proteolytic activity is concentrated in the fraction which contains the protease particles. However, at a suboptimal level of inositol (0.5  $\mu$ g/ml) the protease activity is contained mainly in the soluble fraction, only a small percentage still being located in the position when the protease particles are normally found in the density gradients.

These findings lead to the conclusion that a shortage of inositol results in insufficiently tightened protease particles and subsequent release of proteases into the cytoplasm. Since in homogenates from suboptimally cultured mycelia incubated at 28°C a much more rapid breakdown of protein occurs than in extracts from normally grown mold, it seems to be very likely that the fr. proteases initiate the autolysis of the cytoplasm. In germinating conidia cultured in the absence of inositol, the autolysis may become complete due to the absence of septa in germ tubes. At low concentrations of inositol, growth of the hypha proceeds unless the inositol is exhausted. In this case, the degeneration may affect only that part of the hypha which has been formed last (probably the tip); since the autolysis of the cytoplasm results in the liberation of fr. inositol (Fuller and Tatum 1956 *Amer. J. Botany* 43: 361) a further limited growth of the surviving part of the hypha will take place, the repetition of this process leading to the formation of a highly branched small colony. ■ ■ ■ Department of General Botany, Swiss Federal Institute of Technology, Zurich, Switzerland.