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Elucidation of "inositol-less death"

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The phenomenon of "inositol-less death" in Neurospora is the basis of on 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 on 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, th. 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 lad to the detection of a constitutive cytoplasmis particle which contains the proteolytic enzymes to be secreted into protein-containing growth media (Motile 1965 Z. Zellforsch. 65:884). These secretory granules hav. been termed protease particles (Motile 1964 Naturwissenschaften 51:489); they represent small spheres (diameters from 0.15 to 0.3μ) surrounded by a single membrane (Motile et al. 1965 Z. Zellforsch. in press). Incorporation of either choline-C^{14} or inositol-C^{14} into respective heterotrophic :trains followed by cell fractionation (density gradient centrifugation) and analysis of the lipids has shown that the lipid composition of th. membranes of protease particles is significantly different fmm that of other cytoplasmic membranes: they are relatively poor In lecithin and rich In inositol phospholipid.

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

There findings lead to th. conclusion that a shortage of inositol results In insufficiently tightened protease particles and subsequent release of proteases Into th. cytoplasm. Since In homogenates from wboptlmolly 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 th. fr.. proteases initiate th. autolysis of th. cytoplasm. In germinating conidia cultured In the absence of inositol, the autolysis moy become complete due to th. absence of septa In germ tubes. At low concentrations of inositol, growth of the hypha. proceeds unless the inositol Is exhausted. In this co,. th. degeneration may affect only that part of the hypha which has been formed lost (probably th. tip); since the autolysis of th. cytoplasm results in th. liberation of fr.. inositol (Fuller and Tatum 1956 Amer. J. Botany 43:361) a further limited growth of th. surviving part of th. hypho. will take place, the repitition of this process leading to the formation of a highly branched small colony. ▪ ▪ ▪ Department of General Botany, Swiss Federal Institute of Technology, Zurich, Switzerland.