Fungal Genetics Reports

Volume 12 Article 2

Phospholipids in N. crassa

M. S. Kappy

R. L. Metzenberg

Phospholipids in N. crassa

Abstract

Phospholipids in *N. crassa*

Kappy, M. S. and R. L. Metzenberg.

Phospholipids in Neurospora crassa.

The earliest published report on the phospholipid composition of N. crassa came from Ellman and Mitchell (1954 J. Am. Chem. Soc. 76: 4028) who identified a number of nitrogenous bares. Crocken and Nyc (1964 J. Biol. Chem. 239: 1727) hove demonstrated that monomethyl and dimethyl ethanolamine con be major base components of the phospholipids in certain choline-deficient mutants -a fact that demonstrates that, at least with respect to the components they studied, the mtio of phospholipids in Neurospora can be varied over wide limits. We have examined the mole percent composition of the total cellular phospholipids, utilizing a chromatographic method which was kindly mode available to us prior to publication, and which we have found to be simple and highly reproducible. This method involves measurement of the deocyloted products fmm the phosphatides (R. L. Lester, in preparation).

Conidiol suspensions of wild type N. crassa (Oak Ridge genetic background) were prepared as previously described (Trevithick and Metzenberg 1964 Biochem. Biophys. Rer. Commun. 16: 319) and were used to inoculate cultures which were grown at 25°C in Fries minimal medium (Ryan, et al. 1943 Am. J. Botany 30: 784) containing ^{32}P-labelled inorganic

phosphate at a total concentration of 1 mM. The mass increase during the growth period was of such a magnitude that the cells may be regarded as uniformly labelled. Sucrose (1.5%) was used as the carbon source. The relative amounts of seven identifiable deacylated phosphatides were measured at different stages of culture growth up to "full growth" or the stationary phase. Growth was assessed by measuring the dry weight of duplicate cultures in each instance.

The results are shown in Table 1 and include a normalization of total cellular lipid phosphorus to another cellular constituent, namely, total RNA. It can be seen that there are definite trends in the mole percent composition of cellular phospholipids as the culture "ages". It is also evident that the amount of lipid phosphorus markedly increases in proportion to RNA as full growth is attained.

When the phospholipid composition of a mutant with permeability defects and other alterations of membrane function (mutant 55701 t, also known as un-t (55701) was examined, no differences could be found at a given stage of growth when compared to wild type. However, at all stages of growth, the mutant had significantly less lipid phosphorus per mg. of RNA. It should be noted that the mutant grows more slowly than does the wild type and that at a given chronological age there is a distinct difference between the ratios of various types of phospholipids in the mutant as compared to the wild type. We consider these differences as artifactual, since they disappear when the strains ore compared on the basis of physiological age. Additional studies showed no differences between the mole ratios of phospholipids in the two mating types of Neurospora.

Table 1. Average mole percent composition of cellular phospholipids (+ 1 S.E.$_M$) at different stages of growth of wild type strain.

Deacylated phosphatide	24% grown	59% grown	100% grown
L-α-Glycerophosphate	4.2 ±0.4%	5.6 ±0.4%	9.0 ±0.9%
Glycerophosphorylinositol	10.9 ± 0.8	10.6 ± 0.6	11.0 ± 0.8
Glycerophosphorylserine	3.Bf0.3	6.6 f0.4	11.0 ± 0.8
Glycerophosphorylethanolamine	34.4 ± 1.1	31.6 ± 1.3	30.6 ± 1.3
Glycerophosphorylcholine	41.2f0.9	38.7 ± 2.0	30.8 ± 1.7
Glycerophosphorylglycerol	1.0 ± 0.1	0.8 ± 0.1	0.6 ± 0. 1
Diglycerophosphorylglycerol (deacylated cordiolipin)	4.4 ± 0.6	6.0 ± 0.6	7.0 ± 0.5
Total lipid phosphorus (mμM/mg RNA)	42.Bf3.0	62.8 ± 5.0	114.3 ± 5.5

This research was carried out during the tenure of one of us (MSK) of a predoctoral fellowship of the American Cancer Society (Grant #PRE-6) and was also supported by a U.S. Public Health Service Research Career Development Award (K3-M-19416). The laboratory work was supported by a Public Health Service Grant (GM-08995-05). * * * Department of Physiological Chemistry, University of Wisconsin Medical School, Madison, Wisconsin 53706. Present address MSK: Department of Pediatrics, University of Colorado Medical Center, 4200 E. 9th Avenue, Denver Colorado 80220.