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Lack of RNase-sensitive RNA-dependent DNA polymerase activity in the nuclear fraction of Neurospora crassa 74A

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Abstract

Lack of RNase-sensitive RNA-dependent DNA polymerase activity in the nuclear fraction of *Neurospora crassa* 74A

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RNase-sensitive RNA-dependent DNA polymerase activity (RDDP) has been reported earlier (Dutta, Beljanski and Bourgarel (1977) Exptl. Mycol. 1:173-182) in the post-mitochondrial fraction of N. crassa mycelia. We were unable to detect this RDDP activity in the purified nuclear fraction of this fungus. The nuclear franction was isolated and purified using the procedure described earlier (Hautala, Conner, Jacobson, Patel and Giles (1977) J. Bact. 130:704-713). Minor adaptations to their procedure were made. The french pressure cell containing germinated conidia was placed in an ultrafreezer at -70° C for 1.5 hours instead of immersing the cell in an equilibrated dry ice-ethanol bath for 25 minutes, and the gradients were centrifuged at 8,500 rpm in an SW 25.2 rotor for 35 minutes. The pure nuclear pellet was suspended in two volumes of buffer containing 50 mM Tris HCl pH7.9, 10 mM $MgCl_2$, 5 mM Dithiothreitol (DTT), 0.1 mM EDTA and 40% V/V glycerol. Purify of nuclei was tested by staining with 4,6-diamino-2-phenyl-indole as described by Hautala et al. (1977) using a fluorescent microscope.

Table 1

Summary of RNase-sensitive RNA-dependent DNA polymerase activity in N. crassa cell fractions.

Reaction	p moles of ^{3}H-dATP incorporated per 100 µg proteins	
	Nuclear pellet	Post-mitochondrial pellet
Complete	1.2	11.00
RNase	1.2	4.30
DNase	0.0	0.0

This activity was obtained from crude proteins before any purification of enzyme was obtained. The low incorporation in the nuclear pellet could be due to the fact that in these studies optimum conditions for reactions given were made for RNase-sensitive RNA dependent DNA polymerase.

Results of tests for RNase-sensitive RNA-dependent DNA polymerase activity (RDDP) in nuclear and post-mitochondrial fractions of germinated conidia are summarized in Table I. The 0.2 ml reaction mixture for the assay of RDDP activity contained 5 mM Tris HCl pH 7.9, 2 mM $MgCl_2$, 0.2 mM $MnCl_2$, 0.1 M NaCl, 5 mM NaF, 5 mM DDT, 2 nmoles each of dNTPs, ^{3}H-dATP (50,000 cpm) and nuclei equivalent to 100 µg protein. Whenever needed, the enzyme was preincubated with RNase (20 µg) at 37° C for 10 min before the addition of dNTPs. The radioactive product was precipitated with 5% trichloroacetic acid (TCA). The precipitate was collected on Whatman GF/A or millipore filters (.45 µm, HA), washed with cold 5% TCA and counted in 10 ml scintillation fluid. All data were corrected for background acid precipitable radioactivity at zero time. In control experiments no ^{3}H-DNA product was found when the enzyme from bath the sources (nuclear and post-mitochondrial) was incubated with DNase (20 µg) at 37°C for 10 min before and/or after the addition of dNTPs. In case of RNase treatment a 60-70% reduction in the TCA precipitable product was observed when the post-mitochondrial fraction was used, whereas no such inhibition was found when extracts from the nuclear pellets were used. (This work supported in part by a contract with the U.S. Department of Energy.) - - - Department of Botany, Howard University, Washington, D.C. 20059.