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Dependence on ribosome production on protein sythesis

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Abstract

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Russell, P.J. and E.A. Hall. Dependence on ribosome production on protein synthesis.

We hove been interested for some time in the regulation of cytoplasmic ribosome biosynthesis in N. crassa and here we discuss some experiments which show that when protein synthesis is inhibited, the maturation of ribosomal RNA (rRNA) and the production of riboromer is severely impaired.

In ititial experimentr we grew duplicate wild type cultures at 25° C to early-log phase and then added cycloheximide (100 µg/ml) to one. At various times thereafter, samples from both cultures were taken and incubated for 5 min in the presence of ^{3}H-lysine. The sample was homogenized with an equal volume of ice-cold TCA, allowed to precipitate for 1hr at 0° C, collected on a GF/C filter, washed with 5% ethanol and incubated overnight of 60° C in a scintillation viol with 0.2 ml 60% perchloric acid and 0.4 ml H_2O_2. After cooling, scintillation fluor was added and the radioactivity in the samples was determined by liquid scintillation counting. The rate of protein synthesis in the cycloheximide-treated culture was reduced to less than 5% of the control culture within 30 min after addition of cycloheximide.

We then studied the effects of cycloheximide-induced inhibition of protein synthesis on the kinetics of synthesis of the 2.4 x10^6 ribosomal-precursor-RNA (pre-rRNA) and the processing of this molecule to mature 25S and 17S rRNAs. (A proposed scheme for rRNA maturation is given in Russell et al. (1976) J. Bacteriol. 127:785. Here we grew wild type at 25°C to mid-log phase and then to the experimental culture we simultaneously added ^{3}H-uridine and cycloheximide to final concentrations of 10 µCi/ml and 100 µg/ml, respectively. The control culture received only ^{3}H-uridine. At specific time points samples of the cultures were token, RNA was extracted and purified using phenol:chloroform:isoamyl alcohol and two ethanol precipitations. The RNA was analyzed by electrophoresis in 2.5% acrylamide-0.125% bisacrylamide:0.5% agarose gels as described by Russell et al. (J. Bacteriol. (1976) 127:785). After electrophoresis the gels were scanned spectrophotometrically, sliced into 1-mm fractions and the radioactivity determined by liquid scintillation counting. For the control culture we found that pre-rRNA was synthesized and processed normally to the 1.27 (25S) and 0.7 (17S) million dalton (Mdal) mature rRNAs. By contrast, in the cycloheximide-treated culture, we observed an apparent accumulation of the 2.4-Mdal precursor and of the 1.4-Mdal RNA that we showed previously to be an intermediate between the precursor and 25S rRNA. Very little 25S and 17S rRNA was produced in the presence of cycloheximide.

Since some mature rRNA was produced under protein synthesis inhibition conditions, we conducted experiments to show whether or not there molecules appeared in riboromer. Here we added 100 µg/ml cycloheximide to a growing culture and after 30 min added 1 µCi/ml ^{3}H-uridine. Eleven hours later the culture was harvested, riboromer were isolated and purified and analyzed by sucrose density gradient centrifugation. The results are consistent with the fact that labeled rRNA become associated with ribosomal subunits albeit at a much reduced level than in a control culture.

In conclusion, the inhibition of protein synthesis by cycloheximide appears to block almost completely the production of mature rRNA from the 2-4-Mdal pre-rRNA. The few mature rRNAs that ore produced do associate with ribosomal subunits. Thus continuing protein synthesis is necessary for ribosomal production in N. crassa, a conclusion that has also been drown from work with other eukaryotes. - - - Deportment of Biology, Reed College, Portland, Oregon 97202.