Loo, M.W.S. A temperature-sensitive mutant of Neurospora defective in ribosome processing (rip-I).

Temperature-sensitive (t) mutants were isolated by the inositoless-death enrichment technique, during a search for phase-specific conidial germination mutants. Although no phase-specific mutants were found among 64 (t) isolates, there were mutants in which both conidial germination and mycelial growth were arrested at temperatures above 33° C. These were screened for defects in macromolecular synthesis by monitoring their incorporation of radioactive precursors into DNA, RNA, and protein at 20° C and 37° C (for labeling procedures, see Loo 1975 J. Bacteriol. 121: 286).

One mutant seemed particularly interesting because its rote of RNA synthesis declined rapidly in cultures shifted to 37° C after 4 hours of conidial incubation at 20° C. Within 1/2 hour after the shift, the rate of RNA synthesis fell to 60% of the preshift rate. The rate of protein synthesis in the mutant increased immediately after the shift to 37° C, but also decreased after longer incubation at 37° C. When conidio of the mutant were incubated directly at 37° C or only incubated at 20° C for 1 hour before the shift to 37° C, there was no apparent inhibition of either RNA or protein synthesis at 37° C. When 16-20 hour cultures of the mutant were shifted from 20° C to 37° C, a more rapid inhibition of RNA synthesis than protein synthesis was again observed. It seemed that the (t) lesion in this mutant affected RNA synthesis initially, but hod no effect on freshly incubated conidia. The work of Bhagwat and Mahadevan (1970 Mol. Gen. Genet. 109: 142) and Mirkes (1974 J. Bocteriol. 117: 196) suggested that early conidial proteins might be translated off prepackaged messenger RNA (mRNA), making mRNA synthesis dispensable early in conidial germination. Since the inhibition of RNA synthesis was never complete, it seemed possible that the mutant was defective in the transcription or processing of a single RNA species, mRNA.

The synthesis of translatable mRNA at 37° C was monitored in two ways: by the assay of inducible enzyme activity, and by the sedimentation analysis of newly mode mRNA. Turner, Sorsoli, and Matchett (1970 J. Bocteriol. 103: 364) hod demonstrated that the induction of the tryptophan degradation enzyme kynureninase probably occurred at the transcriptional level, and that it could be monitored by the excretion of anthranilic acid into liquid media. Our (t) mutant, rip-l, showed a more rapid induction of anthranilic acid excretion at 37° C than at 20° C, nearly identical to the induction kinetics of its progenitor strain. This suggested that neither the synthesis nor the translation of new mRNA was appreciably impaired at the restrictive temperature.

This idea was supported by the sedimentation analysis of RNA at 37° C. Cultures were shifted to 37° C after various periods of growth at 20° C, and labeled RNA procursors were added at the time of the shift. Cell extracts were either centrifuged in sucrose gradients containing a high concentration of magnesium to cause the dissociation of ribosomes into subunits, or were subjected to phenol extraction so that purified RNA could be centrifuged in sucrose gradients. Both kinds of preparations indicated that the mutant mode abnormally low levels of 25s riboromal RNA; which is part of the large ribosomal subunit, at the restrictive temperature. Figure 1 shows the profiler of labeled RNA mode during the first 2 hours of conidial incubation at 37" C by the inl parent and rip-l (the mutant). The former was grown in the presence-of ^{14}C-uracil (100 μCi/50 ml, 60 mCi/mM), and the latter in the presence of ^{3}H-adenine (500 μCi/50 ml, 17 Ci/mM). Cell lysates were combined, and phenol-extracted and centrifuged together. The ratio of labeled 25s to 17s RNA was 1 .3 for the progenitor strain, and only about 0.4 for strain rip-l. A similar, though less revere, deficiency of 25s RNA made at 37° C was observed in RNA extracted from strain rip-l after a shift to 37° C. It may be noted that label incorporation by whole cells during the first 2 hours of incubation at 37° C was originally thought to be normal, because there was an increase in the conversion of ^{14}C-uracil into a form insoluble in 5% trichloroacetic acid. It is possible that RNA degradation fragments account for this discrepancy between the labeled RNA seen in whole cells and that observed in cell extracts.

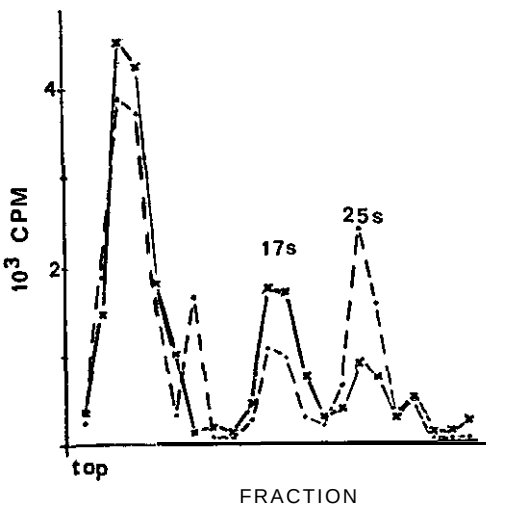

Figure 1. Sedimentation profiles of RNA extracted from conidio after 2 hours of incubation at 37° C. Newly mode RNA was labeled with ^{14}C-uracil in the progenitor strain inl a (---·--) and ^{3}H-adenine in the mutant strain rip-1 (-X-).

Warner and Udem (1972 J. Mol. Biol. 65: 243) hove characterized the processing of riboromal RNA in yeast. There are many steps in which the loss of function of a protein could interfere with the production of isolatable 25s RNA. These include transcription, cleavage, methylation, transport, and riborome assembly. The identification of rRNA precursors in mutant rip-l would aid greatly in pinpointing the defective processing stage. No precursors were obvious in sucrose gradients of extracted RNA of the mutant. However, labeling period were sufficiently long that rapidly degraded precursors would not have been seen. Clearly, much work remains to be done on the molecular characterization of mutant rip-l.

The genetic characterization of this mutant is also incomplete. The (t) defect was recessive in nutritionally forced heterokaryons, as judged by the ability to grow at 37° C. It segregated as a single gene mutation in tetrads. Tetrad analysis also indicated that the rip-l mutation was distant from any centromere. The location of rip-1 is not yet known, but crosses have failed to show linkage to the following genes: al-2 (IR), m.t. (IL), arg-5 (IIR), ad-2 (IIIR), col-4 (IVR), inl (VR), lyr-1 (VC), ylo-1 (VIL), and met-7 (VIR).

Mutant rip-l has been deposited in the Fungal Genetics Stock Center collection and is available for studier on riborome processing in Neurospora. ■ ■ ■ This work was done at the University of Washington, under the guidance of David R. Stadler. -- current address: Deportment of Physiological Chemistry, University of Wisconsin, Madison, Wisconsin 53706.