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P. J. Russell
Reed College

S. C. Schlitt
Reed College

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Rates of RNA and protein synthesis in the cold-sensitive mutant, crib-l

Abstract

Rates of RNA and protein synthesis in cold-sensitive mutant

Russell, P. J. and S. C. Schlitt. Rates of RNA and protein synthesis in the cold-sensitive mutant, crib-I.

The cold-sensitive mutant strain crib-I has been shown to synthesize disproportionate amounts of cytoplasmic ribosomal subunits at 10°C: instead of the 2.3:1 moss ratio of 60S:37S ribosomal subunits characteristic of wild type, the mutant strain exhibits a mass ratio of approximately 7:1 (Schlitt and Russell 1974 J. Bacteriol. 120: 666-671). The aim of the experiments reported here was to compare

the rates of RNA and protein synthesis in the wild type (74A) and the crib-I strain to determine whether or not the mutant strain is conditionally defective in the synthesis of either or both of these classes of macromolecules.

The rates of in vivo RNA and protein synthesis were determined by incubating myceliol pods in the presence of [5-^{3}H]uridine and [4,5-^{3}H]-lysine, respectively, and measuring the amount of trichloroacetic acid (TCA)-precipitable radioactivity after selected time intervals. For both strains tested, 125-ml Delong flasks containing 30 ml of liquid Vogel's minimal medium were inoculated to a final concentration of 10^6 conidia per ml and incubated without shaking at 25°or 10°C until a myceliol pad had formed on the surface of the medium. At this time the labeled precursor was added to a final concentration of 0.5 μCi/ml, and the cultures were shaken for selected time intervals (5 to 60 min) at the appropriate temperature. Mycelial pods were harvested on filter paper with vacuum filtration and dried by passing large quantities of acetone through the myceliol mat. Each dried pad was weighed and then homogenized in 5 ml of 0. 1 M potassium phosphate buffer, pH 7.8. The homogenate was clarified by centrifugation, the supernatant liquid was brought to 5% TCA, mixed thoroughly and allowed to stand for 20 min at 25°C. The resultant precipitate was collected by centrifugation, washed, and resuspended in 5 ml of water. The particulate suspension was mode homogeneous by the addition of NaOH and then the radioactivity in each sample was determined by liquid scintillation counting.

Fig. 1 compares the rates of in vivo RNA synthesis for wild type and crib-I at 25°C (the pemissive temperature) and at 10°C (the nonpermissive temperature). The data indicate no significant difference in the rots of RNA synthesis for the two strains when incubated at 25°C. However, at ICPC the rote of RNA synthesis in crib-I is substantially lower than that of wild type; indeed, in the interval between 30 and 60 min, it is approximately 4% that of wild type.

Fig. 2 compares the rates of in vivo protein synthesis far the two strains at 25°and 10°C. At 25°C there is little difference. By contra+, at 10°C a slight decrease in the rate of amino acid incorporation into protein is observed for crib-I compared with wild type. However, this difference in rates of protein synthesis is not as marked as the difference in rates of RNA synthesis of the two strains.

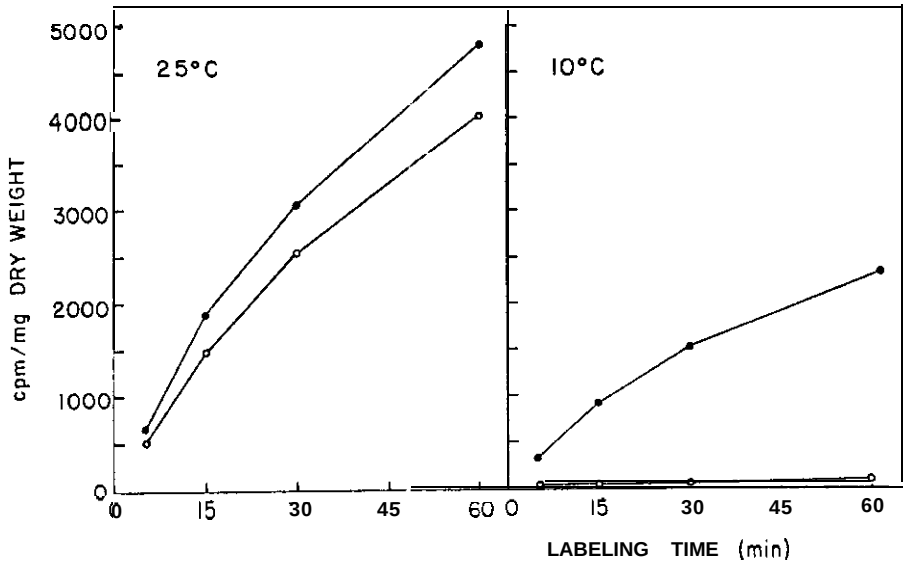

Figure 1. The rates of [5-^{3}H]uridine incorporation into RNA by wild type (●) and by crib-I (O) at 25° and 10°C.

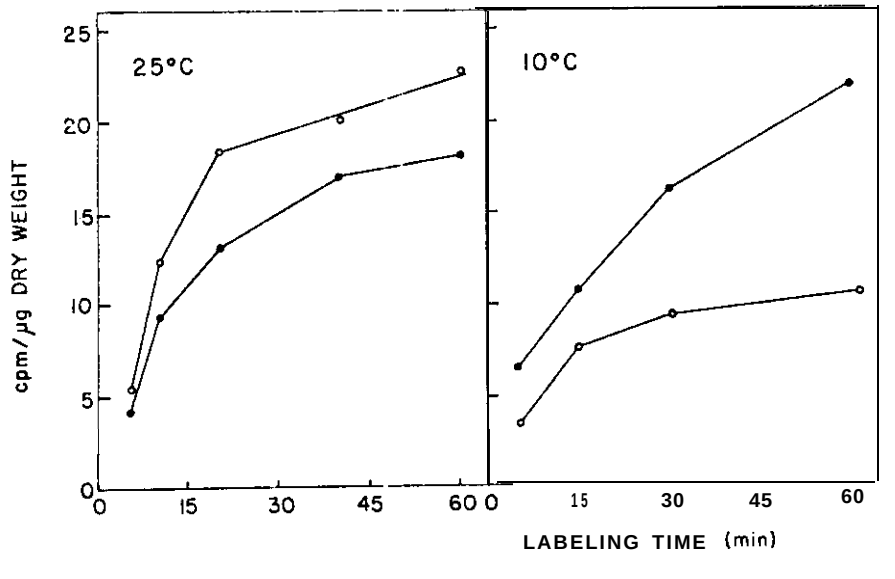

Figure 2. The rates of [4,5-^{3}H]lysine incorporation into protein by wild type (●) and by crib-I (O) at 25° and 10°C.

These results suggest that the primary functional defect in the crib-I strain is in the synthesis and/or accumulation of RNA and that the observed decrement in the rate of protein synthesis at 10 C is a secondary consequence of that defect. Since the largest fraction of the cytoplasmic RNA is rRNA, the primary lesion in crib-I may involve the production of rRNA. In eukaryotes, 3 of the 4 species of rRNA are produced from a common, high-molecular-weight precursor RNA molecule which is specifically methylated and cleaved to result in the mature species. Since the crib-I strain is also characterized by a disproportionate accumulation of ribosomal subunits, we hypothesize that crib-I has a conditional defect in the production or accumulation of 17S rRNA from the high-molecular-weight precursor molecule. - - - Biology Department, Reed College, Portland, Oregon 97202.