JOURNAL OF BACTERIOLOGY, Mar. 1976, p. 1112-1119
Vol. 125, No. 3

Printed in U.S.A.

Neurospora crassa Cytoplasmic Ribosomes: Cold-Sensitive Mutant Defective in Ribosomal Ribonucleic Acid Synthesis

PETER J. RUSSELL,* JAMES R. HAMMETT,[1] AND ERIC U. SELKER[2]

Biology Department, Reed College, Portland, Oregon 97202

Received for publication 22 December 1975

Experiments were conducted to characterize further the biochemical defects of *crib-1* (PJ30201), a cold-sensitive mutant strain of *Neurospora crassa* with a defect in ribosome biosynthesis. The results are as follows. (i) The critical temperature for the expression of the mutant growth and ribosome phenotypes is in the range of 18 to 20 C. (ii) No preferential breakdown of 37*S* cytoplasmic ribosomal subunits synthesized by *crib-1* at 25 C occurs after a shift to 10 C. (iii) Ribosomal subunits synthesized by *crib-1* at 25 C function normally in in vivo protein synthesis at 10 C. (iv) Whereas wild type synthesizes both ribosomal subunits in a coordinate manner after either a temperature shift-down (25 to 10 C) or a shift-up (10 to 25 C), noncoordinate synthesis of ribosomal subunits owing to underproduction of 37*S* subunits occurs in the *crib-1* strain immediately after a temperature shift-down. (v) After a shift from 10 to 25 C *crib-1* exhibits a 12-h lag before the growth rate and the rate of synthesis of 37*S* subunits begin to increase significantly. (vi) At 10 C *crib-1* synthesizes unequal amounts of 25*S* and 17*S* ribosomal ribonucleic acid (rRNA) molecules, resulting from a greatly reduced accumulation of stable 17*S* rRNA. The mutant phenotypes of *crib-1* are proposed to be the result of a defect in rRNA processing.

Recently we reported the isolation and preliminary characterization of *crib-1*, a cold-sensitive mutant strain of *Neurospora crassa* with a defect in ribosome biosynthesis (9). This mutant strain synthesizes a disproportionate ratio of cytoplasmic ribosomal subunits at 10 C: instead of the 2.3:1 mass ratio of 60*S*:37*S* subunits characteristic of the wild type, *crib-1* ribosomal subunits exhibit a mass ratio of about 7.2:1. Ribosomal subunits synthesized by the *crib-1* strain at 25 C are present in approximately wild-type proportions (mass ratio, 3.25:1). Thus, in view of the conditional ribosome phenotype exhibited by the *crib-1* strain, we consider it to be a potentially valuable mutant with which to investigate the biosynthesis of eukaryotic cytoplasmic ribosomes. In this paper we present a further characterization of *crib-1* and provide evidence that this strain is defective in the accumulation of stable 17*S* ribosomal ribonucleic acid (rRNA) molecules at 10 C.

MATERIALS AND METHODS

Strains. The wild-type strain of *N. crassa* used was the St. Lawrence strain *74 A*, further inbred in the laboratory of A. M. Srb (Cornell University, Ithaca, N.Y.) from whom it was obtained. The origin of the cold-sensitive, ribosome biosynthesis mutant *crib-1* (PJ30201) used in this study has been described previously (9).

Culture techniques and growth measurement. The culture techniques, growth media, and the method used to determine lateral mycelial growth rates in growth tubes were all as described by Schlitt and Russell (9).

Buffers and solutions. The following buffers and solutions were used: (i) buffer C (9), consisting of 0.01 M tris(hydroxymethyl)aminomethane (Tris), 0.5 M KCl, 0.001 $MgCl_2$ (pH 7.8 at 4 C); (ii) TEBS, consisting of 0.01 M Tris, 0.05 M ethylenediaminetetraacetate, 0.5 mg of bentonite per ml, 0.5% (wt/vol) sodium dodecyl sulfate (pH 7.0 at 4 C); (iii) TPE (6), consisting of 0.036 M Tris, 0.03 M NaH_2PO_4, 0.001 M ethylenediaminetetraacetate (pH 7.8 at 25 C). The [5-^{3}H]uridine (8 Ci/mmol) and [4,5-^{3}H]lysine (50 Ci/mmol) used for labeling cultures were obtained from Schwarz/Mann, Orangeburg, N.Y.

Growth conditions for ribosome isolation. Conidia were used to inoculate 100 ml of liquid complete medium in a 300-ml Delong flask that was shaken for 48 h at 25 C. The medium was decanted, and the mycelial growth was blended with 100 ml of minimal medium for 15 s in a sterile, stainless-steel, Eberbach semimicro blender cup (2). The blended contents were transferred to a fresh 300-ml Delong flask. Subsequent treatment of the blended culture varied with the type of experiment, and details are included in the figure legends.

[1] Present address: Biology Department, Lewis and Clark College, Portland, Ore.

[2] Present address: Department of Biological Sciences, Stanford University, Stanford, Calif.

Ribosome isolation and analysis. The procedures used for the isolation of cytoplasmic ribosomes from *N. crassa* were essentially those described previously (9), with the exception that the washed ribosomes were stored frozen at −70 C rather than −20 C.

The ribosomes were examined under high-salt dissociating conditions (4, 7) by zone sedimentation at 4 C in sucrose gradients. Twelve-milliliter, 8 to 20% (wt/vol) linear sucrose gradients were made up in buffer C. Between 3.5 and 4.5 A_{259} units (where 1 A_{259} unit is defined as the amount of 259-nm-absorbing material that yields an A_{259} value of 1 when dissolved in a 1-ml volume) of the ribosome suspensions were layered onto the surface, and the gradients were centrifuged for 5 h at 35,000 rpm in a Sorvall OTD-2 ultracentrifuge, Spinco SW36 rotor. After centrifugation the contents of the gradients were displaced upwards, and 35 fractions were collected from each gradient. For each fraction the A_{259} was measured, and the radioactivity was determined by counting in xylene-based scintillation fluid [0.4% (wt/vol) 2,5-diphenyloxazole, 0.01% (wt/vol) 1,4-bis-2(4-methyl-5-phenyloxazolyl)benzene, 33% (vol/vol) Triton X-114, and 67% (vol/vol) xylenes].

Characteristically a two-peak profile was observed corresponding to the 60*S* and 37*S* cytoplasmic ribosomal subunits (5). For each experiment the ratio of the two subunits was determined by integrating under each absorbance peak (for subunit ratio [optical density, OD]) or under each radioactivity peak (for subunit ratio [counts per minute]). Specific (radio)activity was calculated for each subunit separately by dividing the total radioactive counts in the subunit by the total A_{259} units in that subunit. Specific activity is expressed as counts per minute per A_{259} unit.

In vivo protein synthesis. Cultures of wild type and *crib-1* were grown for 48 h at 25 C in 300-ml Delong flasks containing 100 ml of liquid complete medium. At this time the medium was decanted, and the mycelial growth was blended with 100 ml of fresh minimal medium. The blended cultures were transferred to fresh 300-ml Delong flasks, which were shaken at either 25 or 10 C as desired. To determine the rate of amino acid incorporation into protein after various incubation times, [^{3}H]lysine (1.25 μCi/ml) was added to the cultures 1 h before the proposed harvesting time. Cultures were harvested on cheesecloth, washed with cold water, and homogenized in 10 ml of 0.01 M Tris-hydrochloride buffer, pH 8.0. The homogenate was centrifuged for 10 min at 12,000 × *g* (10,000 rpm), and the supernatant liquid was brought to 10% trichloroacetic acid, mixed thoroughly, and allowed to stand for 30 to 60 min in ice. The resultant precipitate was collected by centrifugation and resuspended in 5 ml of 0.01 M Tris-hydrochloride buffer, pH 8.0. The solution was clarified by centrifuging for 10 min at 12,000 × *g* (10,000 rpm). The protein concentration in the supernatant liquid was determined by the method of Chaykin (3). Radioactivity was determined with 1-ml samples in a xylene-based scintillation fluid.

Preparation of total cell RNA. Midlog cultures were chilled rapidly by adding 3 volumes of ice-cold distilled water. The hyphal growth was collected on glass-fiber filters and then frozen on dry ice. The mycelia were homogenized with 5 ml of TEBS buffer and 0.1 g of carborundum. The homogenate was shaken for 30 min at 4 C with 6 ml of water-saturated redistilled phenol. After centrifugation the aqueous phase was removed and re-extracted with 5 ml of phenol for 10 min at 4 C. The nucleic acids in the aqueous phase were precipitated at −20 C by the addition of 0.1 volume of 20% aqueous potassium acetate and at least 2 volumes of absolute ethanol. The precipitate was collected by centrifugation, dried, and resuspended in distilled water, and the nucleic acids were reprecipitated as before. The resulting precipitate was collected by centrifugation and dissolved in electrophoresis buffer (TPE containing 0.2% [wt/vol] sodium dodecyl sulfate). The amount of nucleic acid in each sample was determined spectrophotometrically.

RNA analysis by acrylamide gel electrophoresis. Cylindrical gels were formed in Pyrex tubing (95 by 5.0 mm) essentially as described by Bishop et al. (1). The gels contained 2.0% (wt/vol) acrylamide and 1.0% (wt/vol) agarose (Sigma), the latter to facilitate handling and slicing.

Electrophoresis was carried out at room temperature, using a Hoefer model DE102 disc electrophoresis apparatus, and used the TPE electrophoresis buffer containing 0.2% (wt/vol) sodium dodecyl sulfate. Gels were subjected to electrophoresis for 30 min at 5 mA/gel before applying the sample to remove unpolymerized acrylamide and ammonium persulfate. Between 10 and 100 μl of RNA sample, containing not more than 25 μg of RNA, was layered on the top of the gel, and electrophoresis was carried out for 2.5 h at 5 mA/gel.

After electrophoresis, the gels were removed from the tubes and scanned at 260 nm using a Gilford recording spectrophotometer equipped with a linear transport. The gels were fractionated into 1.1-mm slices using a Bio-Rad model 190 gel slicer, and the RNA in each slice was hydrolyzed in 0.3 ml of 0.3 N Protosol (New England Nuclear Corp.) containing 5% (vol/vol) water. Radioactivity in each slice was determined in a xylene-based scintillation fluid.

RESULTS

Critical temperature for the expression of crib-1 mutant phenotypes. Previous studies with the *crib-1* mutant strain (9) showed that the mutation it carries is not conditionally lethal. This led us to examine the relationship between the cold-sensitive event and temperature. We compared the effects of different temperatures on the growth rate of strain *crib-1* relative to that of wild type and on the ratio of ribosomal subunits synthesized by strain *crib-1*. As the temperature was raised from 18 to 20 C, a dramatic increase occurred in the relative growth rate of strain *crib-1* (Fig. 1). Moreover, this temperature range was the same as that during which its ribosomal subunit ratio

decreased sharply from that characteristic of this strain at the "nonpermissive" temperature (10 C) to that characteristic of the "permissive" temperature (25 C) (Fig. 2).

Stability of ribosomal subunits after a temperature shift-down. The stability of 60*S* and 37*S* ribosomal subunits was compared in strain *crib-1* and the wild type by pulse-labeling cultures with radioactive uridine at 25 C and chasing with an excess of nonradioactive uridine at 10 C. After 24 h of chase conditions, the specific activity of the *crib-1* 60*S* ribosomal subunit began to decrease markedly, whereas that of the 37*S* subunit remained approximately constant (Fig. 3). During this same time period the 60*S*/37*S* subunit ratio (OD) increased significantly from 3.6 to a value of 7.5, whereas the subunit ratio (counts per minute) remained constant. In a similar experiment with wild type (data not shown), the specific activities of both subunits changed coordinately, decreasing slightly by 24 h and at a greater rate thereafter.

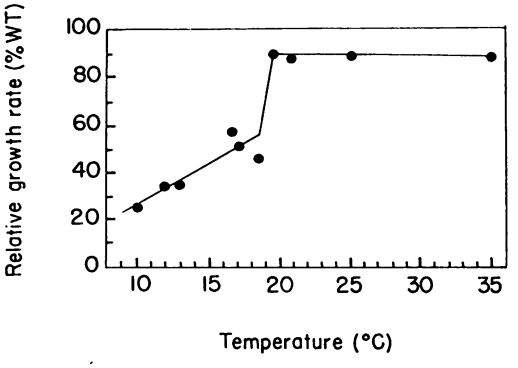

FIG. 1. *Relative growth rate of strain crib-1 as a function of temperature. Lateral mycelial growth rates of the wild type and strain crib-1 were determined in growth tubes as described. For each temperature examined the growth rate of strain crib-1 is expressed as the percentage of the wild-type growth rate at the same temperature.*

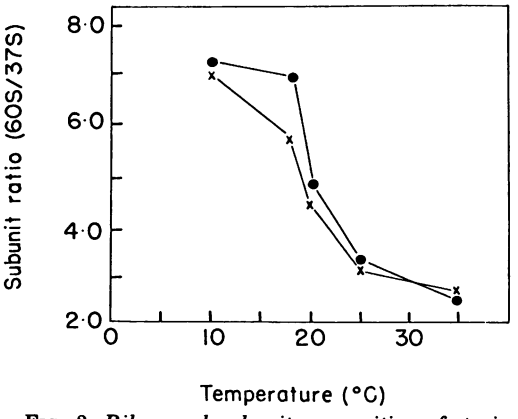

FIG. 2. *Ribosomal subunit composition of strain crib-1 as a function of temperature. Blended cultures were equilibrated for 1 h at the required temperature before labeling for 24 h with [³H]uridine (1.25 μCi/ml). Ribosomes were isolated and analyzed, and the subunit ratios were determined as described in Materials and Methods. Subunit ratio from OD, ●; subunit ratio from radioactivity, X.*

Effects of a temperature shift-down on growth and ribosome biosynthesis. When growth tube cultures of the wild-type and *crib-1* strains were shifted from 25 to 10 C, it was observed that both strains stopped growing for a short time, after which they responded quite differently (Fig. 4). Whereas the wild type quickly established an approximately constant growth rate characteristic of this strain at 10 C, the growth rate of *crib-1* began to decrease at a relatively constant rate, reaching approximately 20% that of wild type 210 h after the temperature shift.

Ribosomal subunit biosynthesis was examined in the wild type and strain *crib-1* by labeling cultures after a shift to 10 C. The specific

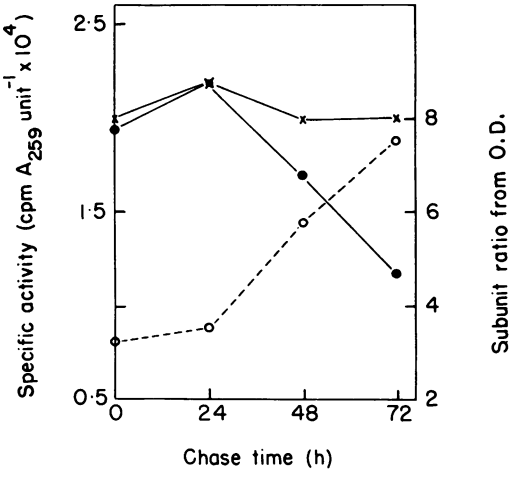

FIG. 3. *Fate of ribosomal subunits synthesized by the crib-1 mutant strain at 25 C after a shift to 10 C. Blended cultures to which [³H]uridine (1.25 μCi/ml) had been added were incubated for 24 h at 25 C, and the mycelial growth from each was collected, washed, and transferred to fresh minimal medium previously equilibrated to 10 C and containing 40 μg of nonradioactive uridine per ml (approximately a 1,000-fold excess over the amount of labeled uridine in the initial culture medium). The cultures were incubated for various times at 10 C, the mycelial growth was harvested, and the ribosomes were isolated and analyzed on sucrose gradients. The zone sedimentation profiles were used to calculate the specific activities and the ratio of the two ribosomal subunits as described. Specific activities: 60S, ●; 37S, X. Subunit ratio from OD, O.*

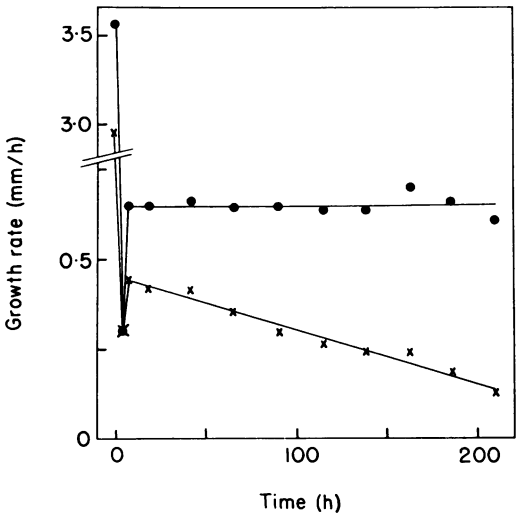

FIG. 4. *Effect of a temperature shift-down (25 to 10 C) on the lateral mycelial growth rate of wild type (●) and of the crib-1 mutant strain (X) determined in growth tubes as described.*

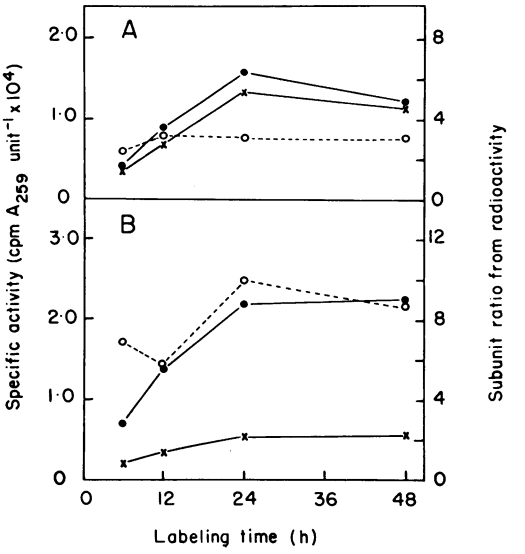

FIG. 5. *Effect of a temperature shift-down on ribosome biosynthesis in the wild type (A) and in the crib-1 mutant strain (B). Blended cultures of each strain were incubated for 24 h at 25 C and then equilibrated to 10 C for 1 h before [^{3}H]uridine (1.25 μCi/ml) was added (except for zero-time cultures). The cultures were incubated for various times at 10 C, the mycelial growth was harvested, and the ribosomes were isolated and analyzed on sucrose gradients. The zone sedimentation profiles were used to calculate the specific activities and the ratio of the two ribosomal subunits as described. Specific activities: 60S, ●; 37S, X. Subunit ratio from radioactivity, O.*

activities of both wild-type subunits increased coordinately in the 24-h period after the temperature shift and reached a maximum at about this time (Fig. 5). During the entire 48-h period examined, the subunit ratio (counts per minute and OD) remained essentially constant. In contrast, synthesis of the *crib-1* ribosomal subunits after the shift-down was not coordinate. The specific activity of the 60S subunit increased at least as rapidly as that of the wild-type 60S subunit increased at least as rapidly as that of the wild-type 60S subunit, but the specific activity of the *crib-1* 37S subunit increased slowly during the first 24 h, at which point it reached a stable level much lower than that of the 60S subunit. The subunit ratio (OD) of the *crib-1* strain increased from 3.2 to a value of 4.0 by 48 h after the temperature shift. That the difference in specific activities of the two subunits was due to disproportionate subunit synthesis is shown by the fact that, within 6 h after the shift to 10 C, the subunit ratio (count per minute) of the *crib-1* strain was approximately 7. This aberrant ratio is due to underproduction of 37S subunits, since the specific activity of this subunit increases very little whereas that of the 60S subunit increases in a manner similar to that of wild type.

Effect of a temperature shift-down on ribosome function. That the ribosomal subunits synthesized by strain *crib-1* at 25 C are functional at 10 C was shown by an experiment in which the incorporation of radioactive lysine into trichloroacetic acid-precipitable material was measured at various times after the temperature shift-down. The results (data not shown) indicated little difference between the incorporation rates for the wild type and strain *crib-1*, at least up to 24 h after the temperature shift. This correlates well with the fact that by 24 h after the shift very little change had occurred in either the growth rate (Fig. 4) or the subunit ratio of the total ribosome population (data not shown) of strain *crib-1*.

Effect of temperature shift-up on growth and ribosome biosynthesis. Temperature-shift experiments were also performed to compare the effects of a shift from 10 to 25 C on growth and ribosomal subunit biosynthesis of strain *crib-1* and the wild type. With regard to the former phenotype, marked differences were observed for the two strains after the shift to 25 C (Fig. 6). The wild-type growth rate increased rapidly for approximately 10 h, after which a roughly constant rate was attained. By contrast, the *crib-1* growth rate remained low for about 10 h before increasing over a 15-h period to a rate about 80% that of the wild type, which is the relative rate characteristic of strain *crib-1* at 25 C.

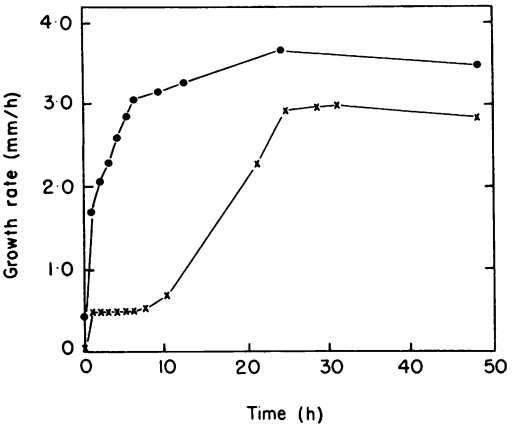

FIG. 6. *Effect of a temperature shift-up (10 to 25 C) on the lateral mycelial growth rate of the wild type (●) and of the crib-1 mutant strain (X) determined in growth tubes as described.*

Ribosomal subunit biosynthesis was examined in the wild type and strain *crib-1* by labeling cultures after a shift to 25 C. The specific activities of both wild-type subunits increased coordinately throughout the period examined, whereas the subunit ratio (counts per minute and OD) remained constant, as expected (Fig. 7). For the *crib-1* strain the specific activity of the 37*S* subunit did not increase concomitantly with that of the 60*S* subunit. Rather, a 12-h lag was observed, followed by a rapid increase until the specific activity reached that of the 60*S* subunit at 48 h postshift. The nature of the newly synthesized (labeled) ribosomes is revealed by the fact that, up to 12 h after the temperature shift-up, strain *crib-1* exhibited a subunit ratio (counts per minute) of about 6.5, which is approximately the ratio characteristic for this strain at 10 C. By 24 h and thereafter, strain *crib-1* synthesized subunits with a ratio of about 3.25, which is characteristic of this strain at 25 C. The subunit composition of the total ribosome population is revealed by the fact that, after a shift to 25 C, the subunit ratio (OD) was observed to decrease from 7.7 at 6 h to 4.4 at 48 h.

rRNA of crib-1. Finally, we did a preliminary analysis of the rRNA's synthesized by strain *crib-1* and by the wild type. When cultured at 25 C, strain *crib-1* synthesized the two major species of rRNA (25*S* and 17*S*) in almost wild-type proportions. In contrast, rRNA synthesized by strain *crib-1* at 10 C was characterized by a disproportionate ratio of 25*S* to 17*S* species, presumably resulting from a greatly reduced accumulation of stable 17*S* rRNA (Fig. 8).

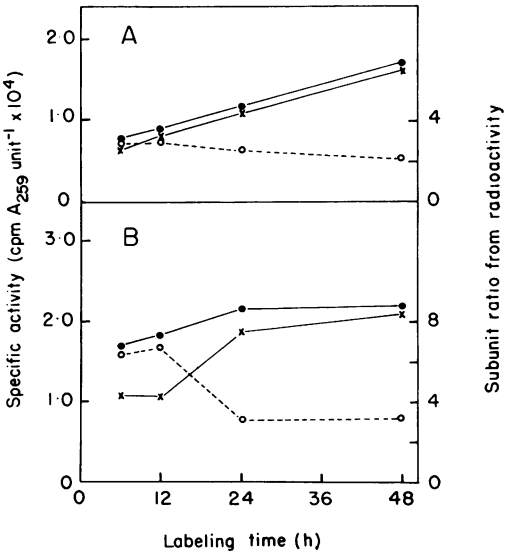

FIG. 7. *Effect of a temperature shift-up on ribosome biosynthesis in the wild type (A) and the crib-1 mutant strain (B). Blended cultures of each strain were incubated for 48 h at 10 C and then equilibrated to 25 C for 1 h before [^{3}H]uridine (1.25 μCi/ml) was added (except for zero-time cultures). The cultures were incubated for various times at 25 C, the mycelial growth was harvested, and the ribosomes were isolated and analyzed on sucrose gradients. The zone sedimentation profiles were used to calculate the specific activities and the ratio of the two ribosomal subunits as described. Specific activities: 60S, ●; 37S, X. Subunit ratio from radioactivity, ○.*

DISCUSSION

The pulse-chase experiments indicate that the specific activities of 60*S* and 37*S* ribosomal subunits synthesized by wild type at 25 C decrease coordinately after a shift to 10 C, whereas the subunit ratio (OD) remains constant. Thus, if any subunit degradation occurs within the duration of the experiment, both subunits are apparently degraded at the same rate. When *crib-1* ribosomal subunits are examined under the same conditions, the specific activity of the 60*S* subunits decreases, whereas that of the 37*S* subunits does not. Furthermore, the subunit ratio (OD) increases significantly by 72 h after the temperature shift-down. These results indicate that new subunits synthesized by strain *crib-1* at 10 C have a high subunit ratio owing to an underproduction of 37*S* subunits, as evidenced by the fact that the specific activity of the 37*S* subunit fails to decrease under chase conditions at 10 C. Therefore, no significant dilution of labeled 37*S* subunits syn-

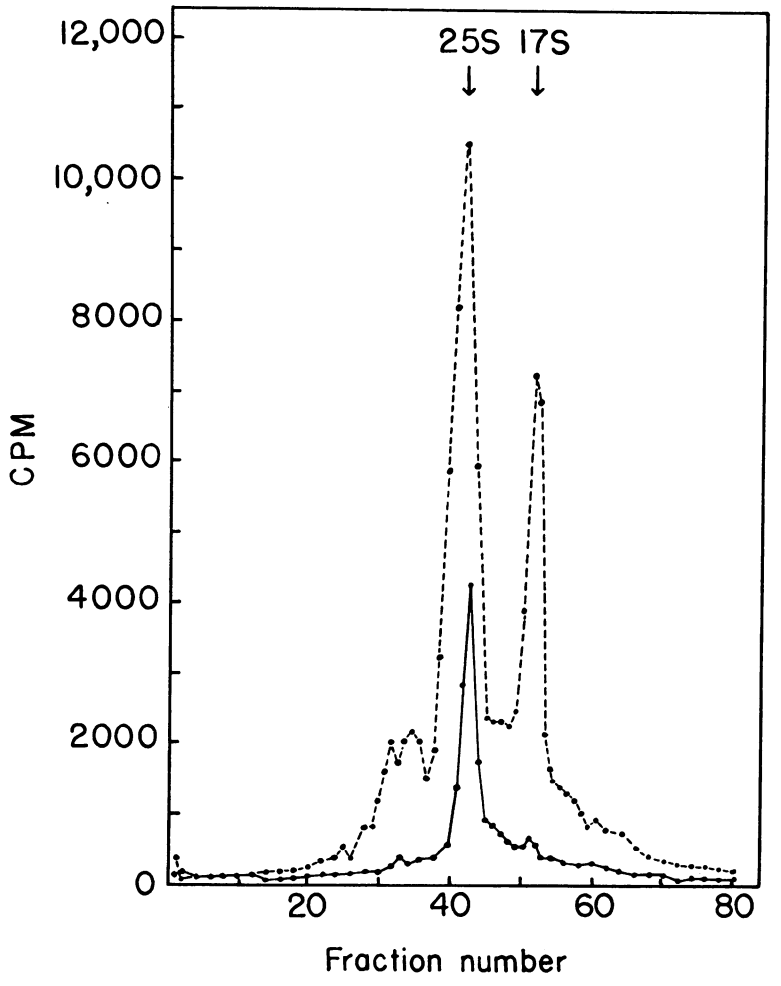

FIG. 8. *RNA synthesis by strain crib-1 and the wild type at 10 C. Separate 300-ml Delong flasks containing 50 ml of liquid minimal medium were inoculated with conidia from strain crib-1 and the wild type and shaken at 25 C for 14.5 h. Then the cultures were equilibrated to 10 C for 1 h with shaking before [^{3}H]uridine was added to a final concentration of 4 μCi/ml. The wild type was harvested after 2 h of labeling and strain crib-1 after 4 h and their RNA was extracted and purified as described. The RNAs were analyzed on 2.0% acrylamide-1.0% agarose gels by electrophoresis for 2.5 h at 5 mA/gel as described. The gels were scanned at 260 nm and sliced into 1.1-mm slices, and the radioactivity in each slice was determined as described in Materials and Methods. The positions of the mature 25S and 17S Neurospora rRNA's were determined from the absorbance pattern and are indicated by the arrows. crib-1, ●——●; wild type, ●-----●.*

thesized at 25 C occurs. Since the subunit ratio (counts per minute) remains constant throughout the chase time, no preferential breakdown of labeled 37*S* subunits occurs after a shift to 10 C. Studies of in vivo protein synthesis correlate well with the growth characteristics of strain *crib-1* after a temperature shift-down in that ribosomal subunits synthesized at 25 C are functionally normal at 10 C.

The study of new ribosome synthesis after temperature shifts shows that both ribosomal subunits are synthesized coordinately in wild type after either a temperature shift-up or shift-down. Thus the assembly of 60*S* and 37*S* subunits from the component protein and RNA molecules in wild-type *Neurospora* must be regulated in a tightly coupled manner. However, this is not the case in the *crib-1* mutant strain. In the temperature shift-down experiment a clear difference in the specific activities of the

two subunits manifests itself within 6 h after the shift. That this is the result of noncoordinate synthesis of new subunits is indicated by the high subunit ratio (counts per minute) at that time, and this, in turn, is due to an underproduction of 37S subunits. By 48 h after the shift the subunit ratio (OD) of the total ribosome population increases relatively little, since synthesis of subunits at 10 C is much slower than at 25 C. And since we have shown that ribosomes synthesized by strain *crib-1* at 25 C are functional in protein synthesis at 10 C, one would expect a relatively small decrease in growth rate of strain *crib-1* by 48 h after the shift, assuming that growth rate is dependent upon subunit composition. Measurements of growth rate proved this to be so.

In the temperature shift-up experiments, the *crib-1* strain exhibits a 12-h lag before synthesis of the 37S subunit and the growth rate begin to increase significantly. In all, about 24 h at 25 C are required before the rate of synthesis of both subunits is the same and before the growth rate becomes stable at a level characteristic of this strain at 25 C. Thus, since (i) new subunits are produced in disporportionate amounts during the first 12 h after either a temperature shift-up or shift-down, (ii) there is no sudden change in the ribosome population present at the time of the shift, and (iii) at least 24 h postshift are required to establish a stable pattern of ribosome synthesis and growth, it may be hypothesized that strain *crib-1* at 10 C has a regulatory or structural lesion early in the synthesis of a component required for ribosome biosynthesis.

It is of interest that the noncoordinate production of 60S and 37S cytoplasmic ribosomal subunits is not unique to the *crib-1* strain. For example, Waldron and Roberts (11) have found four mutants in *Aspergillus nidulans*, all of which produce decreased numbers of 60S ribosomal subunits. Toniolo et al. (10) have shown that a temperature-sensitive hamster cell line is defective in the production of the large, but not small, ribosomal subunits at high temperature. The slow growing, non-Mendelian mutant of *Neurospora*, *poky*, is deficient in the synthesis of small mitochondrial ribosomal subunits (8). The noncoordinate production of ribosomal subunits by the *crib-1* strain at 10 C coincides with the concept of Waldron and Roberts (11), in which the synthesis of the two ribosomal subunits consists of coupled and independent stages.

Preliminary examination of rRNA in order to characterize further the primary lesion revealed that at 10 C *crib-1* synthesize disproportionate amounts of 25S and 17S rRNA species, presumably resulting from a drastic underproduction of 17S rRNA. From this it may be concluded that at 10 C the basis for the abnormal ratio of ribosomal subunits in *crib-1* is the unequal production and accumulation of stable 25S and 17S rRNA molecules, which in eukaryotes are produced by cleavage of a common, high-molecular-weight ribosomal-precursor RNA molecule. Separate studies of rRNA synthesis, in which cultures were labeled immediately after either a temperature shift-up or shift-down, demonstrated that the characteristic rRNA phenotype is expressed immediately after the shift, lending additional support to the interpretation that the defect expresses itself early in the synthesis of the ribosomal subunits. The primary lesion in strain *crib-1*, then, presumably involves rRNA processing and not the assembly of ribosomal subunits. In addition, the loss of coordinate synthesis of ribosomal subunits in strain *crib-1* suggests the existence of a regulatory or structural defect involving the production of 17S rRNA from the ribosomal-precursor RNA molecule.

ACKNOWLEDGMENTS

We thank Steven C. Schlitt for many helpful discussions during the preparation of this manuscript. E. U. S. thanks J. D. Caston and R. Vatev for help with techniques during the early stages of this work.

During the course of this work J. R. H. was supported by a postdoctoral fellowship from the A. P. Sloan Foundation. This research was supported by research grants to P. J. R. from the National Science Foundation (BMS74-18162) and from Research Corporation.

LITERATURE CITED

1. **Bishop, D. H. L., J. P. Claybrook, and S. Spiegelman.** 1967. Electrophoretic separation of viral nucleic acids on polyacrylamide gels. J. Mol. Biol. 26:373-387.
2. **Brody, S.** 1970. Correlation between reduced nicotinamide adenine dinucleotide phosphate levels and morphological changes in *Neurospora crassa*. J. Bacteriol. 101:802-807.
3. **Chaykin, S.** 1966. Biochemistry laboratory techniques. John Wiley and Sons, New York.
4. **Falvey, A. K., and T. Staehelin.** 1970. Structure and function of mammalian ribosomes. I. Isolation and characterization of active liver ribosomal subunits. J. Mol. Biol. 53:1-19.
5. **Küntzel, H., and H. Noll.** 1967. Mitochondrial and cytoplasmic polysomes from *Neurospora crassa*. Nature (London) 215:1340-1345.
6. **Loening, U. E.** 1969. The determination of the molecular weight of ribonucleic acid by polyacrylamide-gel electrophoresis. The effects of changes in conformation. Biochem. J. 113:131-138.
7. **Martin, T. E., and I. G. Wool.** 1968. Formation of active hybrids from subunits of muscle ribosomes from normal and diabetic rats. Proc. Natl. Acad. Sci. U.S.A. 60:569-574.
8. **Rifkin, M. R., and D. J. L. Luck.** 1971. Defective production of mitochondrial ribosomes in the *poky* mutant of *Neurospora crassa*. Proc. Natl. Acad. Sci. U.S.A. 68:287-290.

9. **Schlitt, S. C., and P. J. Russell.** 1974. *Neurospora crassa* cytoplasmic ribosomes: isolation and characterization of a cold-sensitive mutant defective in ribosome biosynthesis. J. Bacteriol. 120:666–671.
10. **Toniolo, D., H. K. Meiss, and C. Basilico.** 1973. A temperature-sensitive mutation affecting 28S ribosomal RNA production in mammalian cells. Proc. Natl. Acad. Sci. U.S.A. 70:1273–1277.
11. **Waldron, C., and C. F. Roberts.** 1974. Cold-sensitive mutants in *Aspergillus nidulans*. II. Mutations affecting ribosome production. Mol. Gen. Genet. 134:115–132.