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Relationship Between Protein and Ribonucleic Acid Synthesis During Outgrowth of Spores of *Bacillus cereus*

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The rate of protein and ribonucleic acid (RNA) synthesis was examined during the outgrowth of spores of *Bacillus cereus* T in a chemically defined medium. RNA synthesis started 2.5 min after the initiation of germination, and protein synthesis after 4 min. Addition of a complete amino acid supplement and uracil supported high rates of RNA and protein synthesis throughout outgrowth. To determine the relationship between the rate of protein (k) and RNA synthesis, the kinetics of formation of various classes of RNA were followed during outgrowth. Ribosomal RNA (rRNA) comprised a relatively constant fraction of the total RNA throughout outgrowth (71 to 78%). The classes of RNA synthesized during this period were determined by germinating spores in radioactive uracil and then at intervals following their stability to actinomycin D. Initially, labile RNA comprised the largest fraction of newly formed RNA (ΔRNA), and this proportion decreased during outgrowth. The ratio of k/rRNA or k/Δ stable RNA varied considerably during outgrowth, whereas the ratio of k/labile RNA remained constant. The data suggest that the rate of protein synthesis is not rigidly coupled to either total or newly synthesized rRNA (ribosomes) during the early stages of outgrowth.

The variation in bacterial composition with growth rate has led to renewed speculation on the regulation of macromolecule synthesis. It has been generally concluded, from studies of exponential cultures and cultures adjusting to a new medium, that the ribosome efficiency (amount of protein synthesized per ribosome per unit of time) is constant at all growth rates (8, 11, 12). This conclusion has recently been modified by Rosset, Julien, and Monier (22), who found that in *Escherichia coli* ribosome efficiency diminishes with decreasing growth rates. Also, the increase in the rate of protein synthesis after a shift-up in *E. coli* (18) suggests that factors other than ribosomes can limit the rate of protein synthesis.

Outgrowth, following the germination of bacterial spores, is analogous to a step-up experiment. Protein synthesis is absent in a dormant spore (14); shortly after germination, ribonucleic acid (RNA) and then protein synthesis commence (1, 2, 14, 25). The correlation between protein and RNA synthesis during outgrowth suggests that the latter determines the rate of protein synthesis. In this study we have attempted to examine this relationship by following the synthesis and labeling of various RNA fractions during outgrowth and relating these to the rate of protein synthesis. Our data suggest that the rate of protein synthesis is proportional to the amount of labile RNA synthesis and not to the amount of total RNA or ribosomal RNA (rRNA) present.

MATERIALS AND METHODS

Preparation of spores. Spores of *Bacillus cereus* strain T were used in this study. Spore crops were collected, washed, and stored as previously described (26). Prior to use, spores (1 to 5 mg/ml) were sus-

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pended in distilled water with a Potter-Elvehjem hand homogenizer, heat-activated at 65 C for 2 to 3 hr, washed once by centrifugation, and suspended in distilled water. Germination was monitored as previously described (26) at the level of 100 to 250 μg of spores/ml.

Media for outgrowth. Chemically defined growth and sporulation medium with glucose (CDGS-Glu) was a modification of that formulated by Nakata (19). The final composition of the medium (in grams per liter) was: L-glutamic acid, 1.8; L-leucine, 0.8; L-valine, 0.3; L-threonine, 0.17; L-methionine, 0.07; L-histidine, 0.050; L-alanine, 0.1; adenosine, 0.5; $CuSO_4 \cdot 5H_2O$, 0.005; $ZnSO_4 \cdot 7H_2O$, 0.005; $MnSO_4 \cdot H_2O$, 0.305; $MgSO_4 \cdot 7H_2O$, 0.412; $(NH_4)_2SO_4$, 2.0; $CaCl_2 \cdot 2H_2O$, 0.007; glucose, 4.0; and potassium phosphate buffer (*p*H 7.0), 0.1 M final concentration.

CDGS-Glu plus supplementary amino acids was prepared by adding the following (in grams per liter): L-serine, 0.6; L-aspartic acid, 0.4; L-arginine, 0.28; L-isoleucine, 0.22; L-tryptophan, 0.1; glycine, 0.1; L-lysine, 0.08; and L-phenylalanine, 0.05.

CDGS-Glu plus supplementary amino acids and bases was prepared by adding a mixture of uracil, cytosine, and guanine (20 μg of each per ml) to CDGS-Glu plus supplementary amino acids.

The other modifications in CDGS medium, and other media employed, are indicated in the Results.

Measurement of protein and RNA synthesis. The incorporation of ^3H- or ^{14}C-amino acids into hot trichloroacetic acid precipitates was used as a measure of protein synthesis; 1 ml of suspension was removed at intervals after the addition of isotope and added to 2 ml of cold 10% (w/v) trichloroacetic acid containing 100 μg (per ml) of nonradioactive amino acid. The samples were heated at 100 C for 15 min, and the precipitates were collected on membrane filters (Millipore; 0.45-μ pore size), washed four times with 5 ml each of 5% cold trichloroacetic acid containing 50 μg of nonradioactive amino acid per ml, and then washed twice with 5 ml of 95% cold ethyl alcohol to remove the acid. The filters were dried overnight in an oven (65 C) and counted in a Packard Liquid Scintillation Counter.

Radioactive uracil incorporation was employed to follow RNA synthesis. The samples were treated as above except that the samples were maintained in ice, the heating step was omitted, and the trichloroacetic acid solutions contained 100 μg (per ml) of unlabeled uracil. Figure 1 summarizes the relationship between phenylalanine and uracil concentration and the rate of their incorporation into protein and RNA. At concentrations above 10 μg/ml, the rates of incorporation of uracil into RNA and phenylalanine into protein were linear and did not vary with concentration. The substrate concentration for half-saturation (K_m) was 2.27 μg/ml for phenylalanine and 3.56 μg/ml for uracil; these K_m values remained constant during outgrowth. Consequently, in subsequent experiments 10 to 20 μg/ml was employed for each to permit quantitative estimates of the amounts of RNA and protein synthesized.

Measurement of total and rRNA synthesis during outgrowth. Heat-activated spores (0.5 g) were inoculated into a 4-liter Erlenmeyer flask containing 2,000 ml of CDGS-Glu medium supplemented with amino acids and uracil and incubated with aeration at 30 C. For total RNA determinations, 1-ml samples were transferred at intervals to tubes containing 3 ml of cold 10% trichloroacetic acid and allowed to stand in ice for 30 min. The precipitates were collected by centrifugation and washed once with cold acid; the pellets were then assayed for RNA by the orcinol reaction. To determine the formation of rRNA, 200-ml samples were removed at intervals, collected on a large Millipore filter, and washed by centrifugation with cold growth medium; the pellets were then frozen. The methods for extraction of RNA and the separation of RNA classes by chromatography on methylated albumin-kieselguhr (MAK) columns are the same as described by Donnellan, Nags, and Levinson (6).

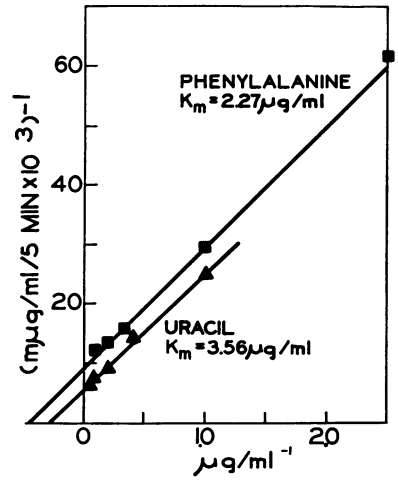

FIG. 1. *Effect of phenylalanine concentration on the rate of amino acid incorporation. Spores were heat-activated and germinated in CDGS-Glu at a concentration of 500 μg/ml. After 30 min of aeration on a rotary shaker at 30 C, 1-ml samples were transferred to test tubes containing either 1.25 μc of ^{14}C-phenylalanine and various amounts of ^{12}C-phenylalanine or 1 μc of ^3H-uracil and various amounts of unlabeled uracil. The tubes were shaken for 5 min and incorporation was stopped by the addition of cold trichloroacetic acid. Zero-time controls were run for each concentration. The radioactivity incorporated into protein or RNA was determined. The inverse of the rate of incorporation (ng per ml per 5 min) is plotted against the inverse of the concentration of substrate (μg/ml). K_m was calculated from the equation: $1/v = (K_m/V_m)(1/S) + 1/V_m$.*

Materials. Actinomycin D, free of mannitol, was kindly supplied by H. B. Woodruff of Merck, Sharp and Dohme Research Laboratories. The following isotopes were obtained from Nuclear-Chicago Corp.: ^{14}C-isoleucine (10 μc/52.4 μg); ^{14}C-serine (100 μc/84 μg); ^{14}C-arginine (100 μc/78.4 μg); ^{14}C-phenylalanine (20 μc/100 μg); ^{14}C-tyrosine (225 μc/181

μg); ^{3}H-lysine (210 μc/146 μg); ^{3}H-uracil (5,000 μc/100 μg).

RESULTS

Onset of protein and RNA synthesis following germination. Two events, germination and outgrowth, precede the reestablishment of vegetative growth. Given optimal germinating stimulants (e.g., L-alanine and adenosine), the rate of germination of spores of *B. cereus* is unaffected by nutrients and by the concentration of spores over the range of 30 to 3,000 μg/ml (Vary, *unpublished data*). To explore the relationship between germination and macromolecule synthesis, spores were germinated in CDGS-Glu medium supplemented with the 20 amino acids and the 4 bases of RNA. The incorporation of excess concentrations of phenylalanine and uracil into protein and RNA, respectively, was examined as a function of time after the addition of the germinating stimulants. The results (Fig. 2) are plotted as counts per minute (uncorrected for zero-time absorption), to illustrate the lag in RNA and protein synthesis. Germination commenced after 2 min and was complete by 6 min.RNA synthesis was detectable after 2.8 min, and protein synthesis was first observed after 4 min.

Also shown in Fig. 2 are the kinetics of uracil incorporation predicted on the assumption that, after an individual spore initiates germination, it rapidly reaches a constant rate of incorporation. Therefore, the rate of incorporation in a suspension is the rate for an individual spore (dP/dt) times the number of spores that initiated germination (A_t). The predicted kinetics parallel the observed incorporation preceding it by approximately 20 sec, which closely approaches the microgermination time in this system (26). To more accurately measure this time, the refractility changes in a single spore were followed as a function of time during germination. The typical germination time for a single spore (Fig. 3) was about 36 sec. Thus, within 25 sec after a spore enters microgermination (Fig. 2), a linear rate of transcription commences.

Conditions affecting outgrowth. The biosynthetic processes occurring during outgrowth are dependent upon enzymes which preexist in the spore and the enzymes synthesized during outgrowth. The duration of this period, as measured by the time required for the first cell division, is influenced by the nutrients supplied and to some extent by the size of the spore inoculum (Table 1). As expected, this time is shortened by the addition of a complete amino acid supplement to the CDGS-Glu medium, and is minimal upon addition of the remaining RNA bases (about 75 min). Not shown in Table 1 is the fact that the effect of added bases can be attributed almost exclusively to uracil addition. Significantly, these variables are also influential in determining the direction in which the emerging cell will develop. Whether the emerging cell will continue outgrowth and divide, or initiate those processes necessary for sporulation (microsporogenesis), is dependent on the available nutrients in the medium.

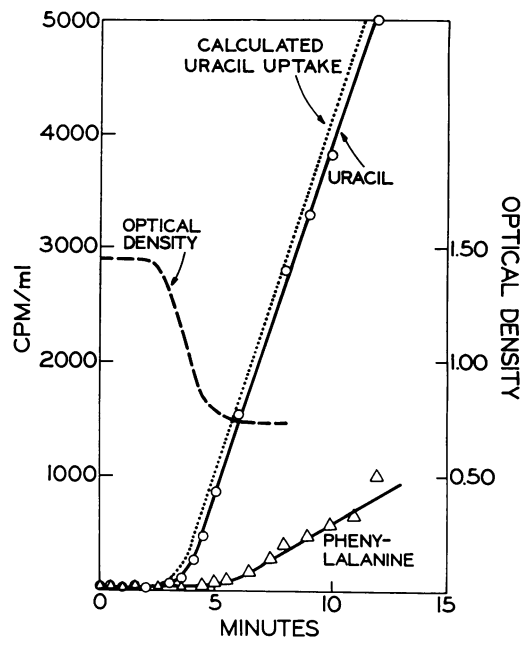

FIG. 2. *Kinetics of RNA and protein synthesis immediately following germination. Heat-activated spores were germinated at 30 C at 500 μg/ml in CDGS-Glu medium supplemented with amino acids and bases and containing either ^{3}H-uracil (5 μc per 46.2 μg per ml) or ^{14}C-phenylalanine (0.1 μc per 23.8 μg per ml). At intervals, 1-ml samples were removed, and the radioactivity incorporated into hot or cold acid-precipitable materials was determined. Germination was measured continuously by following the decrease in optical density at 625 nm in a Gilford Recording Spectrophotometer with the cuvette compartment maintained at 30 C. The theoretical kinetics of uracil uptake were calculated by assuming that dRNA/dt = k (germinated spores), where k = 600 counts/min incorporated per ml per min and the number of germinated spores was calculated from the optical density curve (26).*

The rates of uracil and amino acid incorporation rise early in outgrowth and then decline (14, 27). Such periodicity could be due to (i) differences in the classes of macromolecules synthesized, (ii) limited supply of precursors for

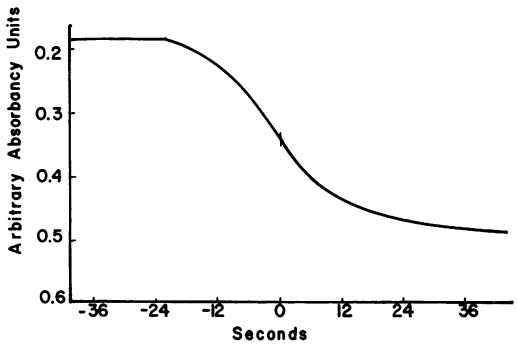

FIG. 3. *Germination of a single spore. The germination of individual spores was observed microscopically (26) with a Zeiss phase-contrast photomicroscope at a magnification of 2,000 ×. A single spore was focused in a restricted field, and the change in refractility was monitored with a Zeiss photometer (Zeiss Bulletin, Microscope Photometer, Micro ZB, 7/67, p. 5, 1967) equipped with a Sargent SRL recorder (0 to 10 mv, 12.7 cm/min). Relative to the background, the absorbancy of an individual spore under phase contrast increases as refractility is lost. That part of the recording which illustrates the timing of germination is plotted as a function of time (seconds) from the midpoint of germination. For germination conditions, see Fig. 2.*

TABLE 1. *Influence of carbon source and size of spore inoculum on outgrowth and sporogenesis*[a]

Medium	Spore inoculum	Time for first division	Type of growth at 15 to 20 hr[b]
	μg/ml	*min*	
G[c]	100	70	I
G	500	75	I
CDGS-Glu	100	220–230	I
CDGS-Glu	500	220–240	I
CDGS-His	50	130–140	I
CDGS-His	500		II
CDGS-glutamate	50	350	I
CDGS-His-Mal	500	240–250	II
CDGS-His-Mal + supplementary amino acids and bases	500	75–80	I
CDGS + supplementary amino acids	250	110–120	I

[a] Spores were heat-activated and germinated at various concentrations in the indicated media. Growth was followed by phase-contrast microscopy, and the time at which the first septum was observed was used as an indication of the first division. An estimate of microsporogenesis was based on the number of cells in the population which contained refractile endospores but had not undergone division. His, histidine; Mal, maltose.

[b] Type I, normal vegetative growth cycle; type II, at least 25% of the culture in microsporogenesis (28).

[c] Modified as previously described (4).

protein or RNA synthesis, or (iii) the onset of a control on RNA synthesis.

To test the first possibility for protein synthesis, the rate of incorporation of six different amino acids was followed by pulse-labeling the cells for a short period of time (5 min) during the first 2 hr of outgrowth in CDGS-Glu medium (Fig. 4). Incorporation of each of the radioactive amino acids into protein was linear during each 5-min pulse. In each case, the rate of incorporation rose for the first 30 to 35 min, then declined sharply, and then increased again in the later stages of outgrowth (after 100 min). With the possible exception of arginine, the kinetics of incorporation of each of these amino acids were essentially the same. Phenylalanine incorporation therefore can be used as an index of protein synthesis throughout outgrowth.

To test the second possibility, that the endogenous reserves and the rates of synthesis of nucleotides and amino acids are insufficient to maintain maximal rates of RNA and protein synthesis, the rates of their formation were followed in supplemented CDGS-Glu media (Fig. 5). The initial rate of RNA synthesis following germination was high (*see also* Fig. 2). Unless the medium was supplemented with added amino acids and bases, the rate rapidly declined during the first hour. The rate of protein synthesis was dependent upon both a complete supplement of bases and amino acids. In CDGS-Glu medium supplemented with bases and amino acids, the rate of phenylalanine incorporation rose continuously for over 1 hr. The decreased rate of protein synthesis after 35 min in unsupplemented medium (Fig. 4) occurred during a period when the rate of RNA synthesis was rapidly falling. This decline was probably due to the limited supply of precursors for RNA synthesis, since in CDGS medium supplemented with amino acids the further increased rate in protein synthesis occurred after this period. Although these results support the second hypothesis, changes in the regulation of RNA synthesis during outgrowth cannot be excluded.

One interesting conclusion from the data in Fig. 5 is that, in a medium which is not completely supplemented with amino acids and bases, there is no direct relationship between the rate of total RNA synthesis and the rate of protein synthesis.

Kinetics of synthesis of protein and different classes of RNA during outgrowth. During outgrowth, the synthesis of all classes of RNA (1, 5, 6) and ribosomal particles (29) has been ob-

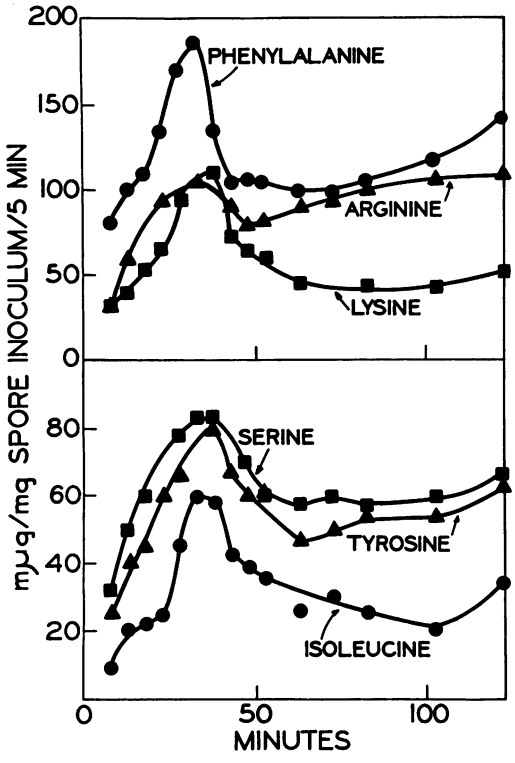

FIG. 4. *Kinetics of incorporation of six amino acids during outgrowth. A suspension of spores (0.25 mg/ml) was germinated in CDGS-Glu medium as described in Fig. 1. At intervals, 1-ml samples were transferred to tubes containing radioactive amino acids and incubated with aeration for 5 min; the reaction was stopped by the addition of cold trichloroacetic acid. The concentration and specific activity of the components of the labeling mixture were: ^{14}C-serine, 0.1 μc per 7.5 μg per ml; ^{14}C-phenylalanine, 0.4 μc per 15 μg per ml; ^{14}C-isoleucine, 0.1 μc per 15 μg per ml; ^{3}H-lysine, 2 μc per 15 μg per ml; ^{14}C-arginine, 0.1 μc per 7.5 μg per ml; and ^{14}C-tyrosine, 0.1 μc per 7.2 μg per ml. At each time interval, zero-time controls were run by chilling the germinated spores in an ice bath, adding the radioactive amino acid, and then immediately adding cold acid. After correction for absorption, the rates of incorporation were calculated from the specific activities and plotted as the function of the midpoint of each incorporation period.*

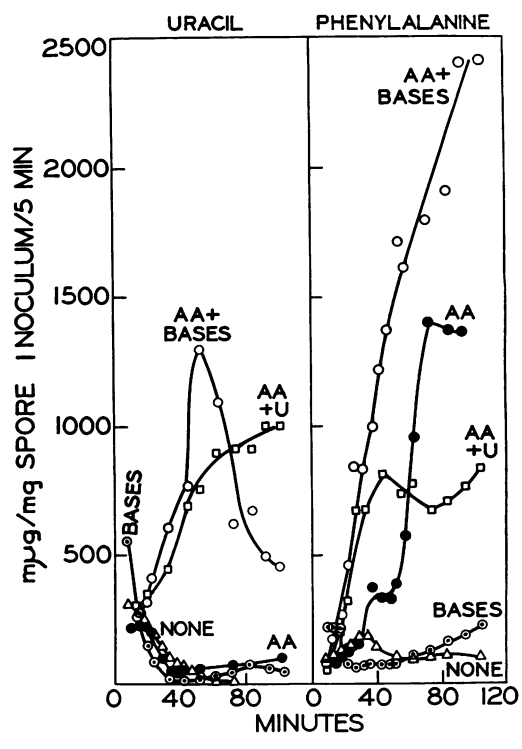

FIG. 5. *Effect of amino acids and bases on RNA and protein synthesis. Heat-activated spores (0.25 mg/ml) were germinated in CDGS-Glu medium supplemented with uracil (20 μg/ml), supplementary amino acids (AA), or bases. At intervals, 1-ml samples were transferred to test tubes containing either ^{14}C-phenylalanine (0.5 μc per 20 μg per ml final) or ^{3}H-uracil (2 μc per 20 μg per ml). After 5 min of aeration at 30 C, the reaction was terminated by the addition of cold trichloroacetic acid, and the incorporation into RNA and protein was measured.*

served. To explore the nature of the dependence of protein synthesis on RNA synthesis, the classes of RNA synthesized during early and late outgrowth were examined in CDGS-Glu medium supplemented with amino acids and uracil. When actinomycin D is added to this system, RNA synthesis stops immediately and labile RNA is degraded within a few minutes (14). Guanine and cytosine were omitted from the medium to minimize the neutralization of actinomycin D.

Figures 6 and 7 show the kinetics of phenylalanine incorporation and of total, stable, and labile uracil incorporation during the early and late stages of outgrowth, respectively. Initially, over 95% of the total uracil was incorporated into labile RNA (Fig. 6). The proportion of stable RNA rose (Fig. 7) continuously during outgrowth and, after 50 min, approached the percentage characteristic of steady-state growth (16). The concentration of labile RNA increased rapidly over the first 40 min and more slowly during the later stages of outgrowth.

Total RNA rose continuously during outgrowth, increasing fivefold over the first 100 min. From the relative amount of rRNA present in the elution patterns of bulk RNA from MAK columns (73 to 79%), and the total increase in RNA in the culture, the amount of rRNA synthesized during outgrowth was determined

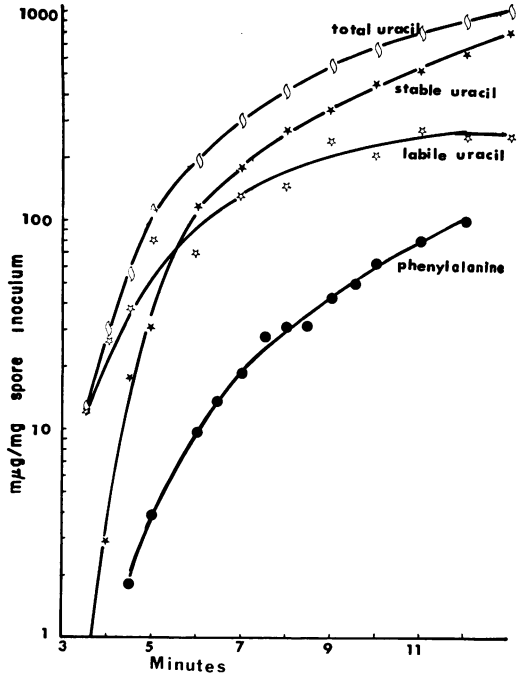

FIG. 6. *Kinetics of RNA and protein synthesis during early outgrowth. Heat-activated spores (500 μg/ml) were germinated at 30 C in CDGS-Glu medium supplemented with amino acids and containing ^{3}H-uracil (2 μc per 20 μg per ml) or ^{14}C-phenylalanine (0.2 μc per 20 μg per ml). For uracil incorporation, two 1-ml samples were removed at intervals. One was added immediately to cold trichloroacetic acid to measure total uracil incorporation. The second was added to a tube containing 10 μg of actinomycin D. The tube was shaken for 20 min in the dark to permit degradation of labile RNA, and the stable cold acid-precipitable activity was determined. Labile uracil was calculated from the difference between total and stable RNA. For phenylalanine incorporation, 1-ml samples were removed at intervals, and the radioactivity incorporated into hot acid-insoluble material was determined. Incorporation was calculated after a correction for absorption was applied.*

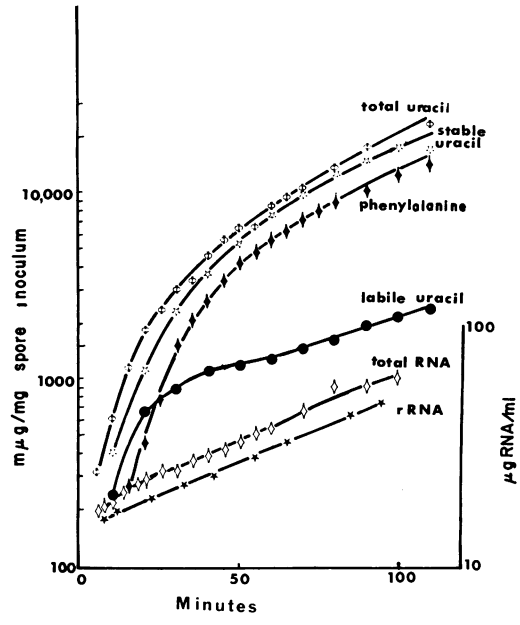

FIG. 7. *Kinetics of RNA and protein synthesis during late outgrowth. Heat-shocked spores (0.25 mg/ml) were germinated at 30 C in CDGS-Glu plus amino acids containing ^{3}H-uracil (2 μc per 20 μg per ml) or ^{14}C-phenylalanine (1 μc per 20 μg per ml). See legend to Fig. 6 for details of uracil and phenylalanine incorporation. Total RNA and rRNA contents were measured at intervals during outgrowth.*

(Fig. 8). The finding that rRNA synthesis commences almost immediately after germination is in agreement with the findings of Fitz-James (9), Woese (29), Balassa (2), and Donnellan et al. (6). The synthesis of soluble RNA (4S + 5S) also commences early as judged from the incorporation of radioactive uracil into these components during the first 8 min after the initiation of germination (Halvorson and Epstein, *unpublished data*).

Relationship between the rate of protein synthesis and RNA content. It is possible to obtain an index of the dependence of the rate of protein synthesis (k) on the total and various RNA fractions during outgrowth by calculating various ratios from the data in Fig. 6 and 7. The ratios of k/rRNA, k/labile RNA, and k/Δ stable RNA are summarized in Fig. 8. When these ratios are plotted as a function of time during outgrowth, striking differences are observed. During the first part of outgrowth, the ratios of k/rRNA and k/Δ stable RNA vary considerably, whereas the ratios of k/labile RNA remain *constant* through outgrowth. If we accept Δ stable RNA as an index of rRNA (the proportion of rRNA remains essentially constant during outgrowth; *see* Fig. 7), then the data strongly suggest that the amount of protein synthesized per unit time is *not* rigidly coupled to either the total or newly synthesized rRNA (and consequently ribosomes) during the early stages of outgrowth.

DISCUSSION

During outgrowth of bacterial spores (conversion to a vegetative cell), not only are all classes of RNA synthesized (1, 5, 6), but protein synthesis is dependent upon the prior synthesis

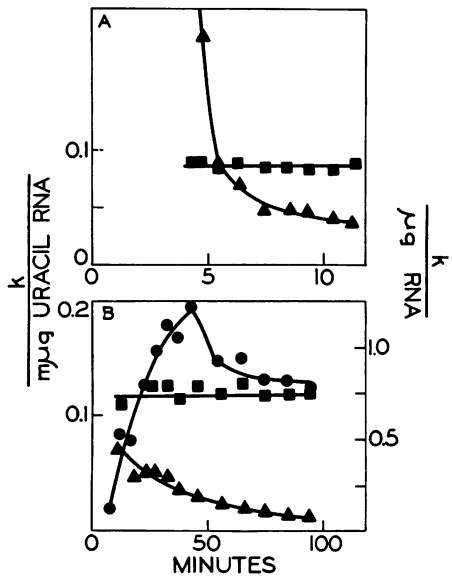

FIG. 8. *Relationship between cell composition and the rate of phenylalanine incorporation during outgrowth. The data are from Fig. 6 and 7. The rate of phenylalanine incorporation (k = ng per min per mg of spore inoculum) was calculated from the kinetics of total phenylalanine uptake. Labile RNA and newly synthesized stable RNA (Δ stable RNA) are calculated as ng of ^{3}H-uracil containing RNA per ml of spore inoculum (left ordinate). The ratios of k/ng labile uracil RNA (■), k/ng Δ stable uracil RNA (▲), and k/μg RNA (●) are plotted (right ordinate) against time during outgrowth. In Fig. 8A, outgrowth was followed from 0 to 12 min; in Fig. 8B, from 0 to 100 min.*

of RNA (for review, *see* 10). Since spores of *B. cereus* T are devoid of stable, functional messenger RNA (mRNA) and after germination contain functional ribosomes, the enzymes necessary for protein synthesis (14) as well as transfer RNA, the developmental changes during outgrowth provide a unique system for examining the relationship between protein and RNA synthesis.

In light of frequent reports that ribosomes are the limiting factor in protein synthesis during steady-state growth, this relationship was first examined during the transition observed during outgrowth. From the data reported here it is evident that neither the total nor newly synthesized ribosomes are functioning at maximal capacity; the efficiency of rRNA varies by over an order of magnitude during the early stages of outgrowth. These results support the conclusions of Rosset et al. (22) that, in vegetative cells under certain environmental conditions, factors other than rRNA can regulate the rate of in vivo protein synthesis.

Changes in ribosomal efficiency would be expected if a variable proportion of the ribosomal population participates in protein synthesis during outgrowth. Dormant spores of *B. cereus* T contain ribosomes which are defective in the polyuridylic acid (poly U)-stimulated in vitro system (13). Also, during outgrowth of spores of *B. subtilis* (29) and *B. cereus* T (13), there is an increase in 70*S* particles and decreases in the amount of smaller ribosomal particles. Since both 70*S* particles (3, 24) and 30*S* + 50*S* particles (21) have each been implicated as the active species in protein synthesis, an analysis of the distribution of ribosomal classes cannot at the moment monitor the number of active particles. However, when ribosome capacity is tested in vitro for phenylalanine incorporation in the presence of poly U, a dramatic transition is observed. Within 5 min after the initiation of germination, and at about the time of the onset of protein synthesis in vivo, ribosome efficiency increases 50-fold to 50% of that from vegetative ribosomes (14). During this period, there is no discernible shift in the classes of ribosomes.

It is generally agreed that ribosome synthesis commences shortly after germination and that it thus could contribute to the supply of functional ribosomes. If the efficiency of *newly formed* ribosomes were the determining factor during outgrowth, then from the present results one would have to conclude that this varies widely during outgrowth.

The constant ratio of *k*/labile RNA throughout outgrowth strongly suggests that it is this species which regulates the rate of protein synthesis. This labile RNA includes mRNA and, in the absence of protein synthesis, unstable ribosome precursors (15). In the early stages of outgrowth in *B. cereus* T (Epstein and Halvorson, *unpublished data*) and *B. subtilis* (1, 6), 4*S*, 16*S*, and 23*S* RNA are all synthesized. The clustering of the cistrons for these RNA species close to the replicating origin of the chromosome of *B. subtilis* (7, 21) might further explain their coordinate synthesis immediately following germination. Although it is possible that translation of ribosomal cistrons prior to the onset of protein synthesis (approximately 3 to 4 min) could contribute to the labile RNA population, several observations lead to the conclusion that the primary active component is probably mRNA. First, during outgrowth, the kinetics of increase in the protein-synthesizing capacity, as measured by the amount of amino acid which can be incorporated after the addition of actinomycin D, closely parallels the increase in labile RNA (Piper and Nickerson, *unpublished data*). Similar correlations were observed by Higa (Ph.D. Thesis,

Massachusetts Institute of Technology, 1964) and Steinberg (Ph.D. Thesis, Univ. of Wisconsin, 1967) under conditions of outgrowth in which the rate of total RNA synthesis did not rise continuously but fluctuated widely. Second, germination leads to the ordered appearance of a number of enzymes, whose syntheses are sensitive to chloramphenicol and actinomycin D (23). Experiments are currently in progress in an attempt to directly determine what proportion of labile RNA is represented by mRNA.

ACKNOWLEDGMENTS

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