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Ribosomal Ribonucleic Acid Synthesis and Maturation in the Blue-Green Alga *Anacystis nidulans*

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Methods are described for preparation of pulse-labeled ribonucleic acid (RNA) from the blue-green alga *Anacystis nidulans*. Synthesis of labeled RNA was found to be in part dependent on concurrent photosynthesis and was inhibited by the antibiotic streptolydigin. Mature 23*S* ribosomal RNA (rRNA) appeared before mature 16*S* rRNA. Formation of either molecule was inhibited by chloramphenicol, and RNA species of lesser mobility accumulated. These species may be precursors of the mature forms. Maturation of 16*S* rRNA was also inhibited by streptolydigin. (The effect of this antibiotic on 23*S* rRNA maturation was not examined). In many respects, ribosomal RNA synthesis and maturation in this blue-green alga appear to follow the pattern already established for bacteria.

Loening (11) and Taylor and Storck (20) have surveyed ribosome structure in the two major groups of prokaryotes, the bacteria and the blue-green algae. Both possess "70*S*" ribosomes composed of two ribonucleoprotein subunits of unequal size. The smaller, 30*S*, subunit carries a single ribosomal ribonucleic acid (rRNA) molecule with a molecular weight of 0.56×10^6 daltons, termed 16*S* rRNA. The larger, 50*S*, subunit carries a single, usually intact, 23*S* rRNA molecule (1.07×10^6 daltons). A third rRNA (5*S* rRNA, 0.04×10^6 daltons) has also been shown, in bacteria, to be associated with the 50*S* ribosomal subunit.

To date, prokaryotic rRNA and ribosome formation, as distinct from structure, have been investigated only in bacteria (13). Pertinent to the present study are the observations that functional bacterial 16*S* rRNA and 23*S* rRNA are each derived from precursors (p16 and p23) of 10 to 20% greater molecular weight (1, 8, 14). Maturation of 16*S* rRNA involves the loss of 50 to 100 nucleotides from each end of the p16 molecule (4, 7, 12, 18), and is sufficiently slow in vivo that p16 is readily detected in pulse-chase experiments. Conversion of p23 to mature 23*S* rRNA is more rapid, and the cleavage event(s) involved is not yet known. Both precursors accumulate (cleavage is blocked) in cells in which protein synthesis is inhibited at the level of translation (1, 13). rRNA precursors substantially larger than p16 and p23 have not been identified in bacteria. In particular, no single large RNA chain containing both 16*S* and 23*S* rRNA sequences has been found (5, 10). This situation is in strong contrast to that in eukaryotes, in which the major rRNA species are both derived from a single, easily detected, high-molecular-weight (41 to 45*S*) precursor (6).

Certain fundamental prokaryotic properties other than the possession of 70*S* ribosomes are shared by bacteria and blue-green algae. Both groups lack defined, membrane-enclosed nuclei or organelles, and both are sensitive to certain antibiotic inhibitors of macromolecular synthesis which do not affect nonorganellar synthesis in eukaryotic cells (19, 23). Detailed investigations of the synthesis of nucleic acids have not been performed with blue-green algae, perhaps because of the difficulty of obtaining these organisms in pure culture (19) and their low permeability to nucleic acid precursors (16). The present report describes a technique for the preparation of pulse-labeled RNA from *Anacystis nidulans*, and presents a preliminary analysis of the pattern of synthesis and maturation of rRNA in this unicellular blue-green alga.

MATERIALS AND METHODS

Growth of organisms. *A. nidulans* was obtained from J. Meyers, University of Texas, and was routinely maintained on slants of the 1.5% agar medium

described by Allen (2). Rigorous aseptic techniques were followed throughout, and the cultures were periodically checked for contamination by (i) microscopic examination, (ii) inoculation into broth (in the dark), and (iii) prolonged incubation on plates as described below. No bacterial contamination was detected. Certain of the experiments were repeated with single clones derived from streaks on agar.

Escherichia coli (B) was grown in broth and labeled for three to four generations with ^{32}P-orthophosphate (Atomic Energy of Canada, Ltd). After one further generation of growth in excess nonradioactive phosphate, RNA was prepared by phenol extraction for use as marker on polyacrylamide gels (14).

Labeling lysis, and RNA extraction. Routinely, 1.8×10^4 cells per cm^2 of surface area were spread uniformly on petri plates containing 1.5% agar in the medium of Allen (2), and were incubated at 37 C, 9 cm above four Westinghouse 20-w Cool-White fluorescent tubes. Under these conditions, growth is exponential with a doubling time of 4 to 5 hr (F. I. Maclean, *personal communication*). After 40 to 50 hr of incubation, cells were washed from the plates in a final volume of 10 ml of 0.5 M mannitol in 0.03 M potassium phosphate, *p*H 6.8 ("mannitol-phosphate"). After 3 to 5 min of centrifugation at 8,000 × *g* at 25 C, cells were resuspended in 1.0 ml of mannitol-phosphate containing 0.001 M ethylenediaminetetraacetic acid (EDTA) and 0.5 mg of lysozyme/ml (Worthington Biochemical Corp.), as described by Pigott and Carr (16), and were swirled gently in the dark at 37 C for 1 hr. The osmotically sensitive cells were then harvested by centrifugation as above, washed once in 5 to 10 ml of the liquid medium of Allen (2) supplemented with 0.5 M mannitol and 0.1% sodium bicarbonate (16), and resuspended in the same medium to a density of between 0.2×10^9 and 1.0×10^9 cells/ml.

For labeling of RNA, 1- to 5-ml portions of this cell suspension were incubated in Pyrex tubes (25 by 250 mm) at 37 C in a glass-bottom water bath illuminated from below by eight General Electric 30-w incandescent "reflector" bulbs at 15 cm. All suspensions were incubated for 10 min before the addition of label, and all were bubbled with 5% CO_2 (in air) throughout the incubation and labeling period. Undiluted uridine-5-^3H (New England Nuclear Corp., ca. 25 Ci/mmole) was added to a final concentration between 0.04 and 0.4 mCi (0.4 to 4.0 μg) per ml. For measurement of ^{3}H-uridine uptake into total nucleic acid, 0.1-ml samples were removed at intervals to 5 ml of cold 5% trichloroacetic acid. The precipitates were collected and washed with cold trichloroacetic acid followed by ethanol on glass-fiber filters, and were counted in a scintillation counter (14). Incorporation into alkali-stable material was measured by trichloroacetic acid precipitation (after neutralization) of 0.1-ml samples incubated with 0.4 ml of 0.25 N NaOH for 24 hr at 37 C.

For lysis and RNA extraction, labeled cells were centrifuged for 3 min at 15,000 × *g* at 0 C, and were resuspended in 4 ml of ice-cold distilled water with the aid of a vortex mixer. A 0.1-ml amount of a 1 mg/ml solution of deoxyribonuclease (Worthington Biochemical Corp., ribonuclease-free) in 0.1 M tris-(hydroxymethyl)aminomethane (Tris)-hydrochloride, *p*H 7.3, containing 0.001 M $MgCl_2$ was added and, after 20 to 30 sec, sodium dodecyl sulfate (SDS; Matheson Co., Inc., recrystallized) was added to 1.0%. This mixture was extracted three or four times with 4 ml of redistilled water-saturated phenol containing 0.1% 8-hydroxyquinoline. After precipitation by the addition of 0.4 ml of 2.0 M sodium acetate and 8 ml of ethanol, RNA was dissolved and analyzed on 2.4% polyacrylamide gels as detailed elsewhere (14).

Chemicals. 3-(*p*-Chlorophenyl)-1,1-dimethylurea was obtained from Dupont, and chloramphenicol, from Sigma Chemical Co. Streptolydigin was a gift of G. B. Whitfield of The Upjohn Co.

RESULTS

Conditions for rapid lysis. Previously, RNA has been extracted from blue-green algae after homogenization or grinding in buffer (11, 20) or after release from a French pressure cell (9). The present analysis requires lysis techniques which minimize the time between termination of labeling and addition of nuclease inhibitors (SDS and phenol). This was achieved by labeling only *after* a preliminary exposure to lysozyme and EDTA, which renders the cells susceptible to rapid lysis.

In Fig. 1 are shown the results of an experiment in which cells were subjected to osmotic shock and SDS treatment after various periods of incubation in mannitol-phosphate buffer containing lysozyme and EDTA. After 60 min of incubation at 37 C (in the dark), cells were maximally susceptible to lysis by osmotic shock and detergent, and a clear green viscous solution was obtained which yielded no visible pellet of intact cells or debris upon centrifugation. Satisfactory lysis could not be obtained if either EDTA or lysozyme was omitted from the incubation mixture, or by repeated freezing and thawing (in the presence or absence of SDS) of unincubated cells.

Incorporation of label after lysozyme treatment. Prolonged (12 to 18 hr) lysozyme treatment has previously been used to produce protoplasts which are photosynthetically active (22) and which show increased ability to assimilate nucleic acid precursors (16). The relatively brief lysozyme treatment used in the present study is insufficient either to convert the cells to protoplasts, as determined microscopically, or to affect the rate of uridine uptake significantly (Fig. 2).

The incorporation of ^{3}H-uridine into trichloroacetic acid-precipitable material by cells treated with lysozyme and EDTA as described and by cells incubated in buffer alone is shown in Fig. 2. A log-log plot was used to spread

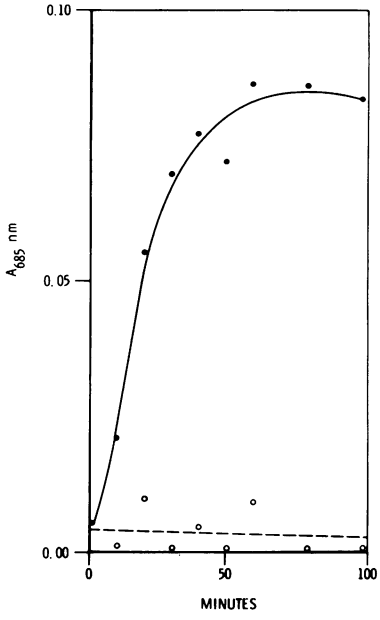

FIG. 1. *Lysis of A. nidulans as measured by release of material absorbing at 685 nm. Cells grown on plates were harvested, washed, and resuspended in mannitol-phosphate buffer* (O) *or in mannitol-phosphate buffer containing lysozyme (0.5 mg/ml) and 0.001 M EDTA* (●). *Samples were removed at the indicated time during incubation at 37 C and were diluted 20-fold in cold distilled water. After the addition of SDS to 1.0%, cells were centrifuged and absorbance due to chlorophyll released in the supernatant fluid was read at 685 nm in a Zeiss spectrophotometer.*

points corresponding to early samples. Incorporation began without a significant lag and was linear with time for at least 1 hr. During this period, approximately 0.05 nmole of uridine was incorporated into trichloroacetic acid-precipitable material per 10^9 cells. This is similar to the values reported by Pigott and Carr for uracil uptake by whole cells of *A. nidulans* (16). It should be noted that the rate of uptake of uridine (as of other low-molecular-weight metabolites) is several orders of magnitude lower than the rates that have been reported for bacteria (3). To eliminate any possible contribution by contaminating bacteria to the results obtained here, aseptic techniques were rigorously observed and the purity of cultures was repeatedly confirmed.

Dependence of label incorporation on photosynthesis. Nucleic acid synthesis should be at least indirectly dependent on photosynthesis in *A. nidulans*, an obligate photoautotroph. Demonstration that this is so is presented in Fig. 3. Shown in Fig. 3 is ^{3}H-uridine incorporation into cell suspensions incubated with 3-(*p*-chlorophenyl)-1,1-dimethylurea) for 10 min prior to the addition of label. This herbicide inhibits only noncyclic electron flow, and should only partially limit energy available for nucleic acid and protein synthesis. ^{3}H-uridine incorporation was more severely reduced in cells in which all photosynthetic activity had been blocked by witholding light (Fig. 3). These results further confirm that the labeling observed was not due to contaminating heterotrophic bacteria.

Preliminary analysis of labeled material. In bacteria, a fraction of label incorporated during short pulses with ^{3}H-uridine appears, as

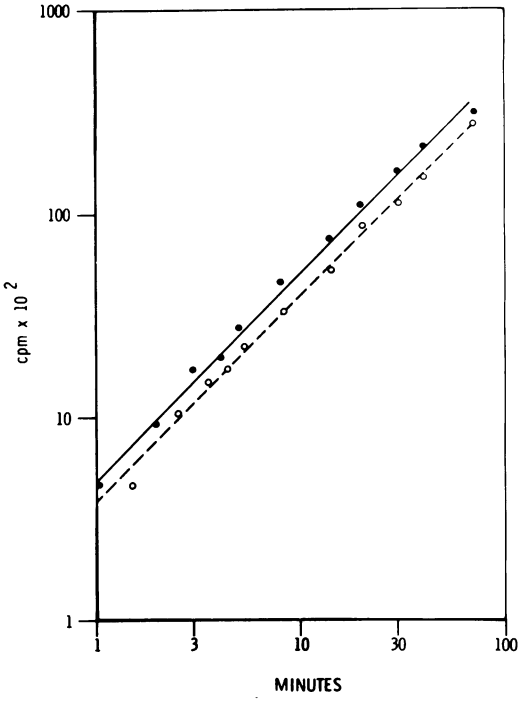

FIG. 2. *Incorporation of ^{3}H-uridine into cold trichloroacetic acid-insoluble material by A. nidulans. Cells were harvested, washed, and resuspended in mannitol-phosphate buffer, or in this buffer containing lysozyme (0.5 mg/ml) and 0.001 M EDTA. After 1 hr of incubation at 37 C, cells were centrifuged, washed, and resuspended in the medium of Allen (2) containing 0.5 M mannitol and 0.1% sodium bicarbonate (2.5 ml, 10^9 cells/ml). After 10 min of bubbling with 5% CO_2 in air at 37 C (with illumination), 0.1 mCi (1.0 μg) of uridine-5-^{3}H was added to each 2.5-ml suspension. Samples were withdrawn at indicated times for trichloroacetic acid plating. The logarithm of trichloroacetic acid-precipitable counts per minute is plotted against the logarithm of time in minutes after the addition of label.* (●) *Cells treated with lysozyme and EDTA.* (O) *Cells treated with buffer alone.*

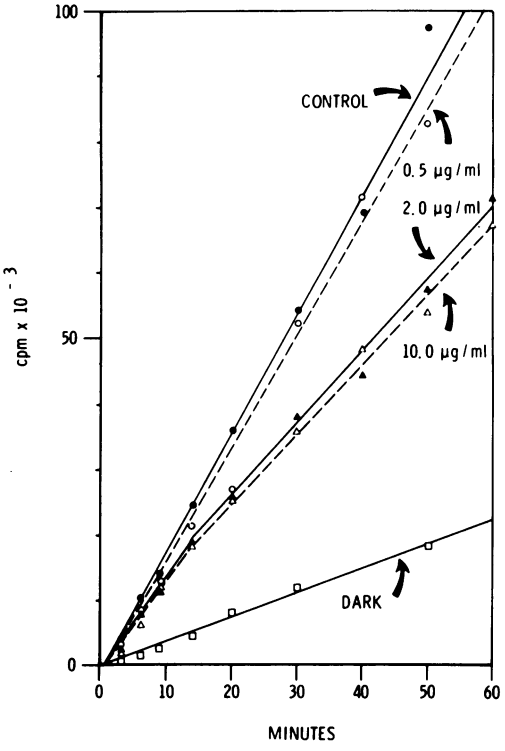

FIG. 3. *Involvement of photosynthesis in ^{3}H-uridine incorporation. A suspension of washed, lysozyme-EDTA-treated cells in medium (2) containing 0.5 M mannitol and 0.1% sodium bicarbonate was divided into five parts. One portion was kept in darkness. 3-(p-Chlorophenyl)-1,1-dimethylurea was added to 0.0, 0.5, 2.0, or 10.0 µg/ml to the illuminated suspensions. All suspensions were bubbled for 10 min with 5% CO_2 in air at 37 C before the addition of label. After addition of ^{3}H-uridine (0.05 mCi/ml), samples were withdrawn for trichloroacetic acid plating as described in Materials and Methods.*

deoxycytidine, in deoxyribonucleic acid (DNA; 3, 15). In the present study, incorporation of label into RNA by *A. nidulans* was distinguished from incorporation into DNA on the basis of (i) alkali lability and (ii) sensitivity to streptolydigin, an inhibitor of transcription (23).

An experiment measuring ^{3}H incorporation into total trichloroacetic acid-precipitable material and material stable to alkaline hydrolysis is shown in Fig. 4. After an initial lag, the rate of label incorporation into stable material was approximately 2% of the total.

Also shown in Fig. 4 is the effect of addition of streptolydigin (to 50 µg/ml) 10 min after the beginning of the labeling period. In bacteria, this antibiotic inhibits RNA chain elongation by direct interaction with RNA polymerase (23). A similar explanation is consistent with the data presented in Fig. 4. The experimental curve can be interpreted as having three components: (i) an unstable component, the decay of which is complete 10 min after the addition of antibiotic, (ii) a stable fraction corresponding to rRNA and transfer RNA, and (iii) a slowly increasing, streptolydigin-insensitive

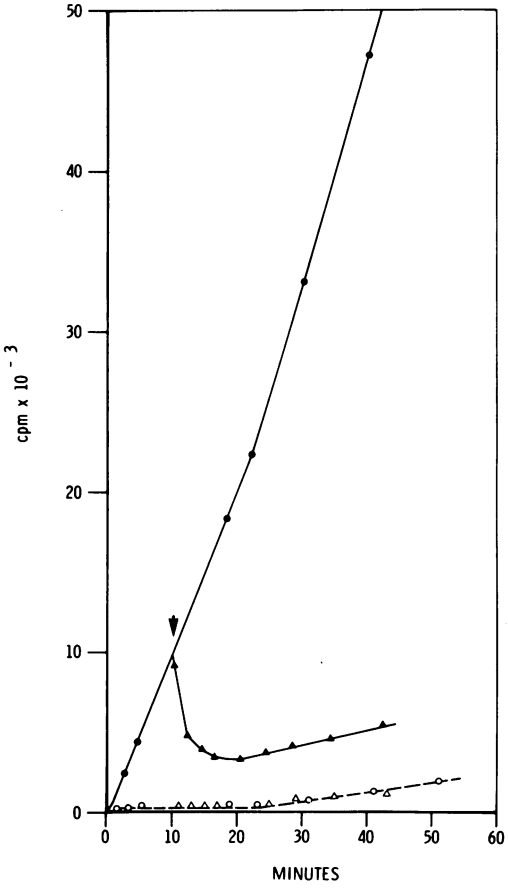

FIG. 4. *Effect of streptolydigin on incorporation of label into alkali-stable and total trichloroacetic acid-precipitable material. Washed lysozyme-EDTA-treated cells were resuspended in medium (2) containing 0.5 M mannitol and 0.1% sodium bicarbonate and were bubbled with 5% CO_2 at 37 C in the light for 10 min before the addition of ^{3}H-uridine (0.05 mCi/ml). Samples were withdrawn to trichloroacetic acid or to 0.25 N NaOH for determination of total or alkali-stable trichloroacetic acid-precipitable material, respectively, as described in Materials and Methods. At 10 min after the addition of label, the suspension was divided into two parts, one of which received streptolydigin (50 µg/ml), and sampling was continued as above. Total trichloroacetic acid-precipitable counts: ●, control; ▲, streptolydigin added. Alkali-stable trichloroacetic acid-precipitable counts: ○, control; △, streptolydigin added. Arrow indicates time of addition of streptolydigin.*

component which is largely alkali-stable and is probably DNA. This analysis is similar to that made by Pato and von Meyenburg (15) for *E. coli* treated with rifampin (which inhibits transcription initiation). However, the fraction of the RNA synthesized during the first 10 min of labeling which is unstable was much greater than would be observed with *E. coli*. This probably reflects a high relative rate of messenger RNA synthesis in *A. nidulans* under the conditions employed (*manuscript in preparation*).

Electrophoretic analysis of labeled RNA. Figure 5 illustrates the kinetics of RNA labeling in a cell suspension exposed to ^{3}H-uridine for 20 min, and then to an excess of ^{1}H-uridine for an additional 40 min. Significant net label incorporation ceased less than 5 min after the addition of nonradioactive uridine. Samples were withdrawn for RNA extraction at 5 (A) and 20 min (B) after the addition of label, and at 10 (C), 25 (D), and 40 (E) min after the chase. The RNA was resolved by electrophoresis on 2.4% polyacrylamide gels, with ^{32}P-labeled *E. coli* rRNA as marker.

The results of this "pulse-chase" experiment allow several conclusions. (i) ^{3}H-RNA is quite heterogeneous in size, even after prolonged labeling. During the chase, much of the heterogeneous material disappears. This, together with the data obtained with streptolydigin (Fig. 4), suggests that a relatively large fraction of the RNA synthesized by *A. nidulans* under the present conditions is unstable. (ii) Late in the chase (Fig. 5C–E), three major peaks of high-molecular-weight RNA were observed on polyacrylamide gels. Two of these were coincident with *E. coli* 23*S* rRNA and 16*S* rRNA, as expected from earlier determinations of the molecular weight of mature rRNA from blue-green algae (9, 11). The third peak had an apparent molecular weight of 0.68×10^{6} daltons and, although more stable than the bulk of nonribosomal material, disappeared slowly during the chase. (iii) The most slowly migrating species labeled in significant amounts at early times in this and other pulse-chase experiments was only fractionally slower than mature 23*S* rRNA. No species of the apparent molecular weight of a possible common precursor of both 16*S* and 23*S* rRNA (at least 1.6×10^{6} daltons) was observed.

Effect of chloramphenicol on labeling patterns. Precise measurement of the kinetics of formation of mature 16*S* rRNA in *A. nidulans* is made difficult by the high heterogeneous background of unstable RNA observed at early times in pulse-chase experiments. In bacteria, chloramphenicol has been effectively used in the study of rRNA maturation (1, 13). When translation is inhibited by this drug, ribonucleoprotein particles accumulate which contain RNA species electrophoretically indistinguishable from the precursors (p23 and p16) of mature bacterial 23*S* and 16*S* rRNA detected in kinetic experiments.

The effects of chloramphenicol treatment on rRNA maturation in *A. nidulans* are illustrated in Fig. 6. [Chloramphenicol is known to be an effective inhibitor of blue-green algal growth (21).] Although no effect on net ^{3}H-uridine incorporation was observed during 45 min of incubation in the presence of this antibiotic at 100 μg/ml, the labeled RNA showed a significantly altered electrophoretic profile (Fig. 6). Whereas the RNA extracted from the control (no antibiotic) cell suspension was largely coincident with marker *E. coli* rRNA, most of the labeled RNA extracted from the chloramphenicol-treated cells migrated either slightly more slowly than *E. coli* 23*S* rRNA, or between 23*S* and 16*S* rRNA. The apparent molecular weight (11) of the latter material was about 0.68×10^{6} daltons. This RNA species may be identical to the relatively unstable labeled material prominent in pulse-chase experiments (Fig. 5).

Effect of streptolydigin on labeling pattern. In bacteria, the formation of mature 16*S* rRNA proceeds when transcription (but not translation) is blocked (1, 13). As shown above, the antibiotic streptolydigin caused immediate inhibition of ^{3}H-uridine incorporation in *A. nidulans*. This is most probably due to blockage of RNA chain elongation, since such a direct effect on transcription is well documented in bacteria (23). Maturation of *A. nidulans* 16*S* rRNA in cell suspensions inhibited by streptolydigin was studied in the experiment illustrated in Fig. 7. In agreement with the previous pulse-chase experiment (Fig. 5), 23*S* rRNA appeared early in the labeling period (Fig. 7A–C) and had largely matured by the time of antibiotic addition (21 min). After inhibition by streptolydigin (Fig. 7D), there was a substantial decrease in the heterogeneity of labeled material, which presumably reflects the decay of unstable RNA species. The formation of mature 16*S* rRNA was inhibited, as it is in chloramphenicol-treated cells. (During a comparable 20-min *chase* period in pulse-chase experiments without antibiotics, formation of mature 16*S* rRNA from material labeled during the pulse was nearly complete). Furthermore, much of the labeled material with an apparent molecular weight of 0.68×10^{6}

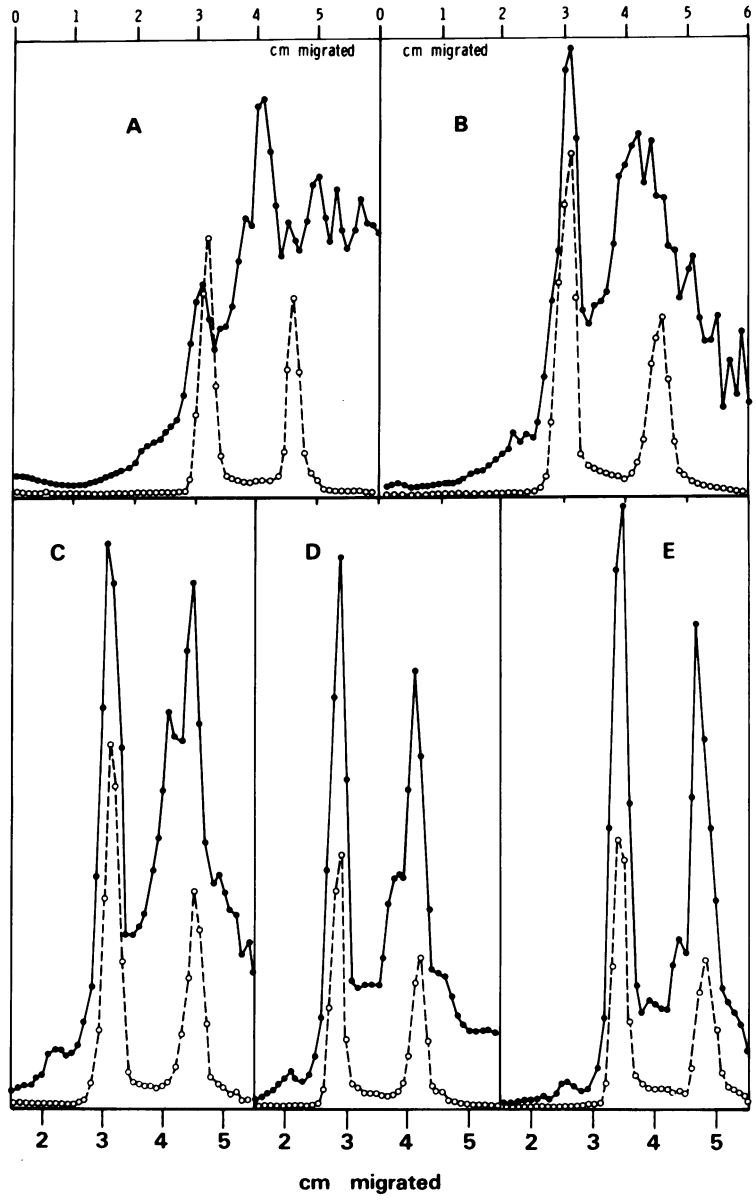

FIG. 5. *Electrophoretic analysis of "pulse-chase" labeled RNA. A washed, lysozyme-EDTA treated cell suspension was labeled for 20 min with ^{3}H-uridine at 0.4 mCi (4 μg) per ml. At 20 min, a 25-fold excess of ^{1}H-uridine was added. Samples were taken for RNA extraction after 5 min (A) and 20 min (B) of labeling, and at 10 min (C), 25 min (D), and 40 min (E) after the "chase." Extracted RNA was mixed with ^{32}P-labeled steady-state E. coli RNA and resolved on 2.4% polyacrylamide gels (14). Different amounts of ^{3}H-labeled material were loaded on each gel, so comparison of absolute counts per minute between gels cannot be made. Arbitrary ordinates were selected to give ^{3}H profiles of comparable height; peak slices on each gel contained at least 10^4 counts/min. (●) ^{3}H-labeled A. nidulans RNA. (○) ^{32}P-labeled E. coli RNA.*

daltons was stabilized by the addition of streptolydigin, as was observed with chloramphenicol.

DISCUSSION

The formation of ^{3}H-uridine-labeled high-molecular-weight RNA in *A. nidulans* under the present conditions showed the following pattern. Material migrating very slightly behind mature 23*S* rRNA on polyacrylamide gels was apparent at the earliest times, and very soon thereafter mature 23*S* rRNA was de-

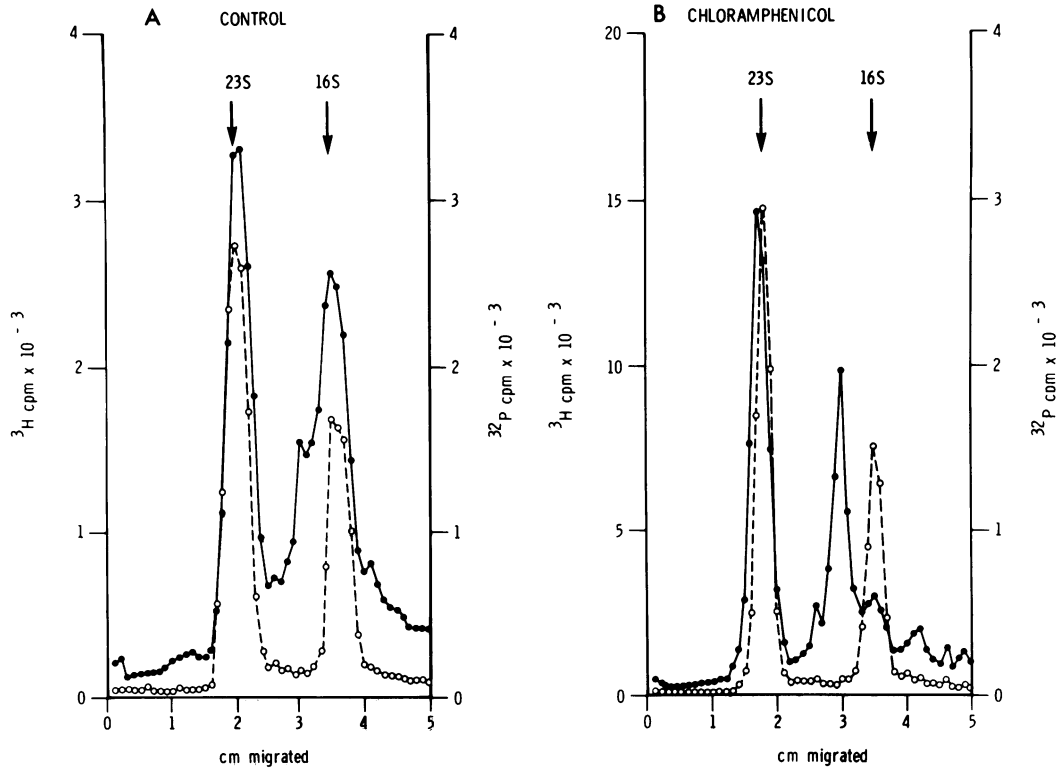

FIG. 6. *Effect of chloramphenicol on rNA maturation in A. nidulans. A washed lysozyme-EDTA-treated cell suspension was resuspended in medium* (2) *containing 0.5 M mannitol and 0.1% sodium bicarbonate and no antibiotic* (A) *or chloramphenicol at 100 μg/ml* (B). *Both suspensions were bubbled with 5% CO_2 at 37 C in the light for 10 min before the addition of ^{3}H-uridine (to 0.1 μCi/ml). RNA was extracted after 45 min of labeling, mixed with ^{32}P-E. coli marker RNA, and resolved on 2.4% polyacrylamide gels.* (●) *^{3}H-labeled A. nidulans RNA.* (○) *^{32}P-labeled E. coli RNA marker.*

tected. There was a similar rapid labeling of heterogeneous material migrating between mature 23*S* and 16*S* rRNA. During a chase with nonradioactive uridine, this material disappeared, although a peak of RNA with an apparent molecular weight of 0.68 × 10^6 daltons persisted until late into the chase. Mature 16*S* rRNA was not observed early in the labeling period. Although detection of this species is made difficult by high backgrounds in the appropriate region of the gels, the results obtained with streptolydigin confirm that mature 16*S* rRNA is not in fact formed as rapidly as mature 23*S* rRNA. A similar difference in the rate of 23*S* and 16*S* rRNA maturation has been observed with *E. coli* (1, 14) and *Bacillus subtilis* (8).

In *A. nidulans*, ribosomal RNA maturation was blocked when translation was inhibited with chloramphenicol. In the presence of this antibiotic, two major rRNA species accumulated, one with mobility only slightly less than that of mature 23*S* rRNA and one migrating with an apparent molecular weight of 0.68 × 10^6 daltons. This is again similar to the pattern observed with bacteria, where, furthermore, kinetic analysis (1, 8, 14) and oligonucleotide fingerprinting (4, 7, 12, 18) have shown the accumulated species to be precursors of mature rRNA molecules which are derived from them by nucleolytic cleavage. In the case of *A. nidulans*, proof that the major RNA species produced in the presence of chloramphenicol are in fact precursors of mature rRNA must await a determination of sequence homology (for instance, by DNA-RNA hybridization). It should be noted that the mobility of the precursor of 16*S* rRNA (p16) in *B. subtilis* is similar to that of the peak observed between 23*S* and 16*S* rRNA in the present experiments (8), whereas that of *E. coli* p16 is somewhat greater (1, 14).

No significant amount of material of the molecular weight expected of a common precursor of both 16*S* and 23*S* rRNA (1.6 × 10^6 daltons or greater) was detected in these ex-

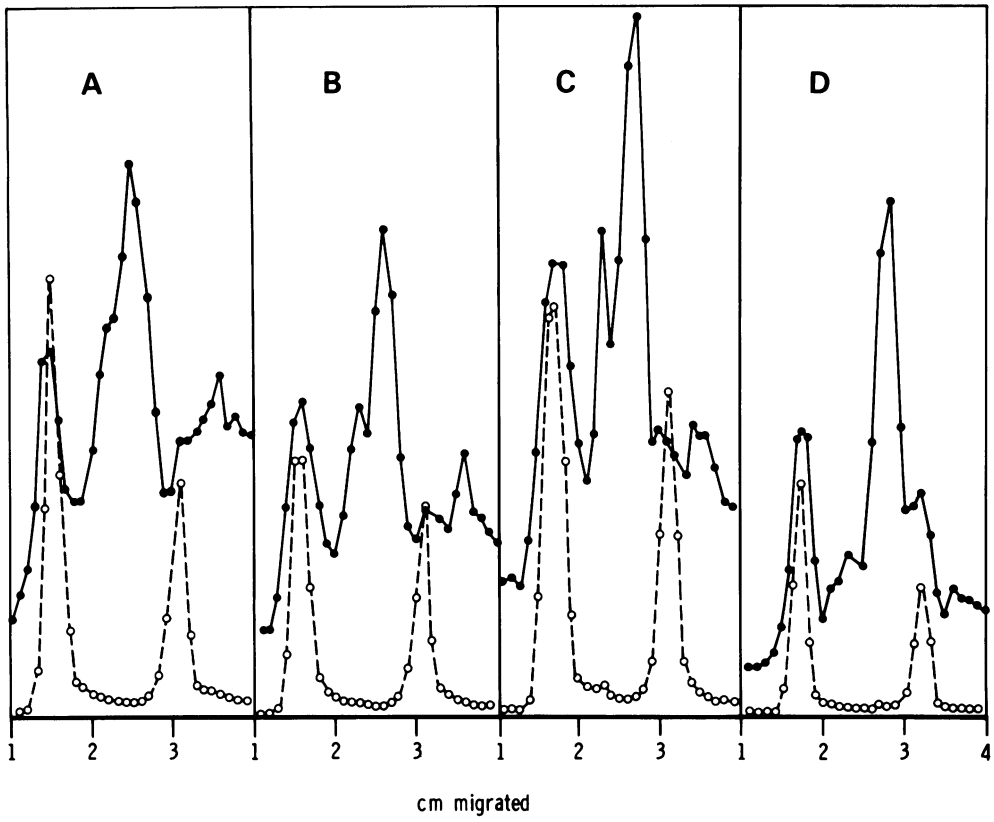

FIG. 7. *Effect of streptolydigin on rRNA maturation in A. nidulans. Washed, lysozyme-EDTA-treated cells were suspended in medium (2) containing 0.5 M mannitol and 0.1% sodium bicarbonate and bubbled for 10 min with 5% CO_2 at 37 C in the light before the addition of label to 0.3 mCi (3.0 μg) per ml. Samples were withdrawn at 7 (A), 14 (B), and 21 (C) min after the addition of label for RNA extraction. At 21 min, streptolydigin was added to 70 μg/ml, and, after 21 min of further incubation with this antibiotic, a fourth sample (D) was withdrawn. Extracted RNA was mixed with ^{32}P-labeled steady-state E. coli marker RNA and resolved on 2.4% polyacrylamide gels. Different amounts of ^{3}H-labeled material were loaded on each gel, so that comparison of absolute counts per minute between gels is not made. Arbitrary ordinates were selected to give ^{3}H profiles of comparable height. (●) ^{3}H-labeled A. nidulans RNA. (○) ^{32}P-labeled E. coli RNA.*

periments. To date, such common precursors have been demonstrated, in vivo, only in eukaryotes, although evidence for transcriptional linkage of 16S and 23S rRNA cistrons in bacteria is accumulating (5, 10, 15).

The maturation pattern observed with *A. nidulans* appears to differ from that obtained with bacteria in that formation of 16S rRNA requires concurrent transcription as well as translation. (The experiments performed do not allow determination of the role of transcription in 23S rRNA maturation.) This observation may suggest that a pool of proteins involved in rRNA maturation (perhaps ribosomal proteins) is very small under the present conditions. It should be noted that these conditions are not "steady-state"; cells were transferred to light only 10 min before labeling, and "balanced-growth" was probably not attained.

It has been suggested (17) that the blue-green algae are closely related to eukaryotic chloroplasts (which have rRNA of the prokaryotic type). Like chloroplasts, blue-green algae must synthesize large numbers of distinct proteins required to maintain the structure and function of the photosynthetic apparatus, and presumably do so in a tightly controlled way. Further investigation of the pattern of nucleic acid and protein synthesis in *A. nidulans* should produce information of both developmental and evolutionary interest.

ACKNOWLEDGMENTS

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