Postmaturational Cleavage of 23s Ribosomal Ribonucleic Acid and Its Metabolic Control in the Blue-Green Alga *Anacystis nidulans*

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Data are presented consistent with the notion that the 23s ribosomal ribonucleic acid (rRNA) of *Anacystis nidulans* undergoes specific endonucleolytic cleavage in vivo, to produce two fragments with molecular weights of $0.88 \times 10^6$ and $0.17 \times 10^6$ daltons. Cleavage occurred at random after 23s rRNA formation and was stimulated by light in this organism, an obligately photoautotrophic unicellular blue-green alga. The half-life of intact 23s rRNA was about 5 h in illuminated cultures and 10 h in unilluminated cultures. 3-(p-Chlorophenyl)-1,1-dimethylurea, an inhibitor of photosystem II, retarded 23s rRNA cleavage in the light. The results are discussed in the context of recent reports of rRNA instability in a variety of eukaryotic and prokaryotic organisms.

Ribosomal ribonucleic acid (rRNA) instability has been demonstrated for both cytoplasmic and organellar ribosomes from a variety of eukaryotic organisms. In most cases, the RNA component (23 to 28s rRNA) of the larger ribosomal subunit appears either to be degraded at a limited number of sites during purification or to contain specific scissions prior to isolation from the cell. This is true of cytoplasmic ribosomes from certain insects (11), molluscs (12), protozoans (21), higher plants (8), and algae (19), and of the chloroplast ribosomes of many higher plants (10, 13). The latter rRNAs yield upon isolation specific fragments, the number and size of which is characteristic of the plant species. Under certain conditions (e.g., in the presence of magnesium ions during extraction and analysis), the fragments remain noncovalently associated and migrate on polyacrylamide gels as a single (23s) RNA species (13). It has been suggested (13, 16) that associated fragments function in vivo in the same way that fully intact 23 to 28s rRNA molecules function and that specific cleavage (although perhaps physiologically important) occurs during ribosomal maturation and aging without loss of subunit integrity.

rRNA lability is uncommon among prokaryotes but has been reported for species of *Agrobacterium* (6) and for the purple nonsulfur bacterium *Rhodopseudomonas spheroides*. The larger ribosomal subunit of *R. spheroides* appears to carry (in addition to 5s rRNA) one rRNA molecule which co-migrates on polyacrylamide gels with 16s rRNA from the smaller ribosomal subunit and a second species 14 to 15s in size (16, 20). Maris and Kaplan (16) have suggested that these high-molecular-weight RNAs are produced by in vivo cleavage of a transient 23s rRNA “precursor,” although other authors consider them to be products of limited specific degradation during extraction (3, 23).

In many instances, it remains unclear whether scission of mature rRNA occurs before or during extraction, and in no case in which it occurs in vivo has the rate of cleavage been measured nor have physiological factors affecting this rate been identified. The present report demonstrates that the 23s rRNA of the prokaryotic unicellular blue-green alga *Anacystis nidulans* undergoes slow postmaturational cleavage to produce two RNA species of unequal size ($0.88 \times 10^6$ and $0.17 \times 10^6$ daltons) and that this cleavage is stimulated by light and retarded by inhibitors of photosynthesis.

**MATERIALS AND METHODS**

*Culture methods.* Wild-type *A. nidulans* was maintained in pure culture on agar slants as described previously (5). Liquid cultures were grown in Allen's (2) medium (7 to 14 ml in a 250-ml capacity flask or 70 to 140 ml in a 2,000-ml flask) with vigorous shaking at 37 C in a New Brunswick G76 gyratory
water-bath shaker illuminated by four 20-W "cool-white" fluorescent bulbs at an average distance of 35 cm from the culture surface. Without added CO₃, such cultures grow logarithmically with a generation time of 4.5 to 5.5 h to a turbidity of 250 units measured in a Klett-Summersen colorimeter equipped with a no. 66 red filter. All experiments were performed with cells in early logarithmic phase (10 to 80 Klett units or ca. 10⁵ to 8 x 10⁷ cells/ml).

Lysis and RNA purification. In a previous report, it was shown that A. nidulans is rendered susceptible to rapid lysis by osmotic shock in the presence of sodium dodecyl sulfate (SDS) after a 1-h exposure to lysozyme and ethylenediaminetetraacetate (EDTA). Although lysozyme-EDTA-treated cells are fully capable of RNA synthesis and maturation (5), high osmotic strength (0.5 M mannitol) is required to prevent them from lysing. For the present series of experiments, intact untreated cells were used for prolonged labeling and chasing, and lysozyme treatment (in the dark) was limited to the hour immediately before lysis, in an effort more closely to approximate physiological conditions.

Typically, cells were harvested by centrifugation at the end of the chase period (0 to 8 h) and resuspended in 0.02 to 0.05 volume of 0.03 M potassium phosphate buffer (pH 6.8) containing 0.5 M mannitol, 0.5 mg of lysozyme ( Worthington Biochemical Corp./ml, and 0.001 M EDTA. After 1 h of incubation at 37 C in darkness, the cells were centrifuged and resuspended in 3 ml of ice-cold distilled water, and deoxyribonuclease (ribonuclease-free, Worthington Biochemical Corp.) was added to a final concentration of 25 μg/ml. The suspension was shaken vigorously on a vortex mixer, after which SDS was added to 1.0% and EDTA to 0.02 M. The resulting lysate was extracted three times with 3 ml of water-saturated redistilled phenol containing 0.1% 8-hydroxyquinoline at 4 C for 10 min. RNA was precipitated from the aqueous phase by the addition of 0.1 volume of 2 M sodium acetate and 2 volumes of absolute ethanol. After overnight storage at –20 C, the precipitate was collected by centrifugation, resuspended in 0.15 ml of E buffer (0.04 M tris(hydroxymethyl)-aminomethane, 0.02 M sodium acetate, 0.001 M sodium EDTA, made pH 7.2 with acetic acid) containing 0.1% SDS, and subjected to electrophoresis on 2.8% polyacrylamide gels in E buffer containing 0.1% SDS as described previously (17). ³²P-labeled Escherichia coli RNA (17) was routinely included as marker during electrophoresis of ³²P-labeled A. nidulans RNA. Gels were sliced, digested with H₂O₂, and counted in Aquasol (New England Nuclear Corp.).

Chemicals. CMU (3-[p-chlorophenyl]-1,1-dimethylylurea) was obtained from Dupont; ³²P-orthophosphate, from Atomic Energy of Canada, Ltd.; and uracil-5,6-³H (40 Ci/mmol) and uracil-5,-³H (23 Ci/ mmol), from New England Nuclear Corp.

RESULTS

Novel RNA species in A. nidulans. Blue-green algae are prokaryotes and possess 70s ribosomes similar in size to those of bacteria (24). The major peaks of rRNA isolated from blue-green algae are coincident with bacterial RNA on polyacrylamide gels. Loening (15) has assigned molecular weights of 1.07 x 10⁶ and 0.55 x 10⁶ daltons, respectively, to the 23s and 16s rRNA of species of Anabena, Nostoc, and Oscillatoria. A recent report from this laboratory has demonstrated, furthermore, that the pattern of maturation of rRNA in A. nidulans is strikingly similar to that observed with bacteria (5). In that study, RNAs coincident on gels with the 23s and 16s rRNA of E. coli were shown to be the predominant stable species labeled with ³H-uridine during relatively short pulse-chase experiments (up to 1 h). It was therefore surprising to find two additional components of stable labeled high-molecular-weight material during repeated attempts to prepare high-specific-activity ³²P-rRNA after prolonged exposure to ³²P-orthophosphate (unpublished data). The two additional RNA species found migrate on gels in the positions expected of RNA species with molecular weights of 0.88 x 10⁶ and 0.17 x 10⁶ daltons (15), and henceforth will be referred to as “0.88M” and “0.17M” RNA, although it should be remembered that molecular weight determinations by other methods have not been made. Together, these findings suggest that, whereas newly synthesized (e.g., pulse-labeled) rRNA is isolatable as the expected 23s and 16s RNA species, “older” RNA (e.g., as isolated after prolonged labeling or after a sufficiently long chase period) is unstable either in vivo or in vitro and is revealed on polyacrylamide gels to have undergone limited specific degradation.

This suggestion was borne out in the experiment illustrated in Fig. 1, in which a single logarithmically growing culture was labeled for four generations with ³²P-orthophosphate, chased for one generation, and then briefly pulsed with ³H-uracil before lysing and extraction of RNA. (Further experimental details appear in the legend of Fig. 1.) The profiles of “old” (³²P) and “new” (³H) rRNA isolated from this culture are substantially different. Old RNA contains much less 23s rRNA and much more 0.88M and 0.17M RNA, whereas the two profiles show similar amounts (25 to 30% of total) of intact 16s RNA. Identical results (not shown) were obtained when the order of label addition was reversed. These experiments must mean that old 23s rRNA does in fact differ in some way from new 23s rRNA in vivo. This difference results in greater apparent fragmentation of the older rRNA as
consistent with the hypothesis that 23s rRNA undergoes in vivo cleavage to produce 0.88M and 0.17M RNA, whereas 16s rRNA remains intact. The kinetic analyses presented require that possible aggregates between the fragments, which might migrate as intact 23s rRNA molecules, be fully dissociated during extraction and electrophoresis. Ingle and others (10, 13) have shown that dissociation of chloroplast rRNA fragments is maximal when RNA extraction and electrophoresis are carried out in the absence of magnesium ions (presence of EDTA). In the current series of experiments, magnesium and other divalent cations were present only in the growth medium and were removed by washing prior to incubation with lysozyme. EDTA at $10^{-5}$ M was included throughout the remainder of the lysis, phenol extraction, and gel electrophoresis operations.

To investigate the possibility that EDTA-stable noncovalently linked aggregates migrating as 23s rRNA were nevertheless present in these preparations, RNA isolated from a culture pulsed for 1 h with $^{3}$H-uracil and chased for 6 h with nonradioactive uracil (sample D of the experiment illustrated in Fig. 2) was subjected to denaturation by heating at 100 C for 0.5, 1.0, or 5.0 min in electrophoresis buffer E (with 0.1% SDS); it was then quickly cooled in ice and loaded onto 2.8% polyacrylamide gels with $^{32}$P-labeled E. coli rRNA as marker. Total recovery of each species was monitored by comparison with the $^{32}$P-marker (Table 1). The nicked duplex DNA contained in the preparation was completely lost after 1 min at 100 C.

![Figure 1](http://jb.asm.org/)

**Fig. 1. Double-labeled RNA.** A logarithmically growing illuminated culture of A. nidulans was labeled for 21 h with $^{32}$P-orthophosphate (10 μCi/ml). The culture was centrifuged, resuspended in fresh medium (without $^{32}$P), and grown for another 5 h before the addition of $^{3}$H-uracil (25 μCi/ml). Uracil labeling was terminated after 45 min by the addition of nonradioactive uracil (100 μg/ml). The "chase" was continued for 1 h, and then cells were treated with lysozyme. RNA was prepared and resolved on 2.8% polyacrylamide gels as described in Materials and Methods. Data are expressed as the percentage of total counts/minute ($^{3}$H or $^{32}$P) retained in the first 7.0 cm of the gel. (●) $^{3}$H; (○) $^{32}$P.

| Time at 100 C (min) | Fraction of remaining label in peak normalized to unit recovery of $^{32}$P-marker RNA*
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*Samples of the $^{3}$H-labeled RNA sample shown in Fig. 2D were heated for 0.0, 0.5, 1.0, or 5.0 min at 100 C, quickly cooled, and mixed with a known amount of unheated $^{32}$P-labeled E. coli rRNA. After electrophoresis, $^{3}$H-material in each of the three high-molecular-weight peaks was determined by summation and normalized to unit recovery of the unheated $^{32}$P-labeled marker 16s rRNA.

*The percentage of the normalized value obtained in the unheated control is shown in parentheses.

Stability of A. nidulans RNA in vitro. Data presented in subsequent sections are most revealed on polyacrylamide gels. Observed variations in relative content of 23s, 0.88M, and 0.17M RNA in short- and long-term labeling experiments are not therefore artifacts of extraction (related, for instance, to changes in nucleic acid levels or ease of lysis and deproteinization with the age of the culture).

Stability of A. nidulans RNA in vitro. Data presented in subsequent sections are most

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**TABLE 1. Heat lability of 23s rRNA, 0.88M RNA, and 16s rRNA**

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whereas more than half of the 23s rRNA component remained intact after 5 min. The material that was lost from this component did not reappear in the 0.88M region, as expected if aggregates were present, and instead was randomly degraded to 16s and smaller fragments.

**Kinetics of in vivo cleavage of A. nidulans 23s rRNA.** The sum of the apparent molecular weights of the 0.88M and 0.17M species identified in "old" RNA is $1.05 \times 10^8$ daltons, which is, within experimental error, the molecular weight of mature *A. nidulans* 23s rRNA. Furthermore, these two novel species are present in approximately equimolar amounts. That is, the ratio of total counts/minute in 0.17M RNA to counts/minute in 0.88M RNA (e.g., 0.17 for Fig. 1) is close to the ratio of their molecular weights (0.19). Therefore, it seems likely that 0.88M and 0.17M RNA are produced as the result of a single specific scission in the 23s rRNA chain.

The pulse-chase experiment presented in Fig. 2 and 3, which shows that the coordinate appearance of labeled 0.88M and 0.17M RNA during the chase is accompanied by the disappearance of equimolar amounts of 23s rRNA, lends strong support to this hypothesis. In this experiment, logarithmically growing cells were exposed to $^3$H-uracil at 20 $\mu$Ci/ml for 1 h, and then the label was chased by the addition of nonradioactive uracil (150 $\mu$g/ml). At various times after the beginning of the chase, samples were withdrawn, treated with lysozyme-EDTA for 1 h (in the dark), and lysed for the preparation of RNA as described in Materials and Methods. RNA was resolved on 2.8% polyacrylamide gels, and the radioactivity in each 1-mm slice is plotted as a percentage of the total radioactivity retained in the first 60 mm of the gel in Fig. 2A-F. ($^{35}$P-labeled *E. coli* RNA was included in each gel, and the positions of 23s and 16s *E. coli* rRNA are marked by arrows in Fig. 2.) It is apparent that the relative amount of 23s rRNA decreases throughout the chase period, whereas 0.88M and 0.17M materials increase in parallel. This relationship is shown more quantitatively by summation of the counts/minute in each component (23s, 0.88M, 16s, and 0.17M RNA) after suitable background subtraction. In Fig. 3, summed counts are normalized so that the amount of 16s rRNA is equal to 1.0 for each time point. Several conclusions can be drawn from Fig. 3.

(i) The 0.88M and 0.17M components accumulate in equimolar amounts. The average ratio of counts/minute in 0.17M to counts/minute in 0.88M is (for the last five time points) 0.21 ± 0.03. (ii) Approximately one molecule of 23s rRNA disappears for every molecule of 0.88M or 0.17M RNA that appears. This is evidenced by the fact that the total normalized counts/minute in "23s derived material" (the sum of counts/minute in 23s, 0.88M, and 0.17M RNA) is essentially constant. (It will be noted, however, that the ratio of total 23s derived material to 16s rRNA is not 2.0, the value expected if both classes of rRNA are obtained with equal yield. It is probable that 50s subunits are preferentially lost prior to deproteinization, since the relative contents of 23s, 0.88M, and
0.17M RNA change coordinately with time even when, as in the experiment illustrated in Fig. 4, the ratio of 23s derived material to 16s rRNA is not constant. (iii) The disappearance of intact 23s rRNA is quite slow, 50% of the labeled 23s rRNA remaining intact at the end of 5 h (ca. one generation under those conditions).

Metabolic control of cleavage. *A. nidulans*, like many blue-green algae, is an obligate photautotroph, and it was of interest to determine whether the cleavage of 23s rRNA pulse-labeled in the light proceeds normally during a subsequent chase period in the dark. For this purpose, a culture was labeled with \(^{3}H\)-uracil (30 \(\mu\)Ci/ml) for 45 min and chased with nonradioactive uracil (300 \(\mu\)g/ml) for an additional 45 min in the light (to allow maturation of precursor rRNA [5]) before one half of the culture was removed to a shielded flask for continued incubation in the dark. Samples were withdrawn from both flasks at intervals, treated with lysozyme-EDTA (in the dark) and extracted as described in Materials and Methods. RNA was resolved on 2.8% polyacrylamide gels, and the total counts/minute in bands corresponding to 23s, 0.88M, and 0.17M RNA were determined by summation as in Fig. 3. In

![Fig. 3. Relative content of high-molecular-weight RNA as a function of time after chase. The total amount of label represented in each of the peaks in the gel profiles shown in Fig. 2 was obtained by summation. The total for each peak was then normalized so that 16s rRNA = 1.0 for each gel. The resulting normalized data are plotted as a function of time after the beginning of the chase. “Total 23s derived material” is the sum of intact 23s, 0.88M, and 0.17M RNA.](image)

![Fig. 4. Cleavage of 23s rRNA in the light (open symbols) and in the dark (closed symbols). A culture was labeled with \(^{3}H\)-uracil (15 \(\mu\)Ci/ml) in the light for 45 min and chased with nonradioactive uracil (150 \(\mu\)g/ml) for 45 min. It was then divided into two samples, one of which was shielded from the light. Samples were withdrawn at intervals from each culture for RNA analysis. RNA was resolved on 2.8% polyacrylamide gels and the percentage of total counts/minute present in each band was determined as for Fig. 3. Data are presented as the percent 23s rRNA remaining intact, i.e., intact 23s + (intact 23s + 0.88M + 0.17M). (○) Dark incubated culture; (■) light incubated culture; (△), data for light-grown culture of Fig. 3 recalculated as above; (□) light and (●) dark incubated cultures of the experiment presented in Fig. 5.](image)
Fig. 4, however, data were not normalized with respect to 16s rRNA and instead, the fraction of total 23s derived material remaining as intact 23s rRNA [intact 23s + (intact 23s plus 0.88M plus 0.17M)] is plotted as a function of time. For comparison, the data of Fig. 3 are replotted in Fig. 4 after calculation in this way.

Scission is substantially retarded in the dark. The apparent half-life of 23s rRNA in the culture without light was 10 h as compared with 5 h in the illuminated culture. There is good agreement between these results and values for the fraction of intact 23s rRNA at various times obtained in other comparable experiments, also plotted in Fig. 4.

In attempting to understand the mechanism of light stimulation of 23s rRNA cleavage, it may be useful to distinguish between possible primary “direct” effects of light on scission (e.g., light-induced changes in the activity of nucleases or structure of ribosomes) and secondary “indirect” effects which depend on functioning of the photosynthetic apparatus (e.g., through alteration in intracellular levels of metabolites affecting nuclease synthesis and activity, or ribosome stability). Such a distinction can be made by using inhibitors of photosynthesis and other cellular functions. In the experiment illustrated in Fig. 5, a culture labeled (45 min, 15 μCi of 3H-uracil/ml) and chased (45 min, 150 μg of nonradioactive uracil/ml) in the light was divided into three subcultures. One subculture was incubated in the light for 6 h with CMU at 10 μg/ml, and control subcultures were incubated in the light and in the dark without additions. RNA preparation and polyacrylamide gel analysis were performed as above, and data are presented in Fig. 5 as the percentage of total radioactivity retained in the first 60 mm of the gel. The proportion of labeled 23s rRNA remaining intact in the illuminated CMU-treated culture (48%) was between the values obtained for the illuminated (34%) and unilluminated (59%) control cultures. CMU, which is an inhibitor of noncyclic photophosphorylation and electron flow, should reduce but not altogether eliminate photosynthetic adenosine triphosphate generation and has been previously shown (5) to inhibit light-stimulated RNA synthesis in A. nidulans by about 40%. The results in Fig. 5 indicate that light stimulation of 23s rRNA scission is in part “indirect,” requiring continued function of the photosynthetic apparatus.

**DISCUSSION**

Studies of rRNA “instability” are plagued by artifacts and misinterpretation. Often it is difficult to determine whether chain scission occurs in vivo or during extraction and subse-
sequent analysis in vitro. Furthermore, the resultant RNA fragments often remain either noncovalently associated after cleavage, or reassociate in vitro and migrate as a 23s aggregate on polyacrylamide gels. In consequence, there exist several reports both confirming (16, 20) and refuting (3, 23) the original claim of Lessie that no stable 23s rRNA can be found in the photosynthetic bacterium *R. spheroides* (14). Similar active controversy surrounds the question of lability of chloroplast RNA from eukaryotic algae (4).

The work with *A. nidulans* reported here allows two definite conclusions concerning rRNA “instability” in this organism. (i) Extracts of cultures labeled and chased for sufficiently long periods contain four major species of high-molecular-weight RNA: the usual 23s and 16s rRNA species and two novel species with apparent molecular weights of 0.88 × 10^6 and 0.17 × 10^6 daltons. The 23s component obtained is quite stable to heat denaturation and thus does not represent an aggregate of smaller species. (ii) RNA labeled early in an experiment (“old” RNA) contains more of the two novel species, and less intact 23s rRNA, than does RNA labeled late in an experiment (“new” RNA). This result cannot be attributed to higher nonspecific nuclease activity and ribosome degradation in older cells nor to some unfortunate and highly repeatable artifact of extraction, since it is true even when the “old” and “new” RNA are extracted together from the same doubly labeled cells (Fig. 1).

While this report was being written, the results of a study of mature and precursor RNAs in *A. nidulans* were published by Szalay et al. (22). These authors presented evidence for the existence of precursors of mature 23s and 16s rRNA analogous to those known for bacteria and in complete agreement with an independent and simultaneous report from this laboratory (5). They also reported finding a novel RNA species equivalent to the 0.88M RNA identified here. This species was found only when magnesium ions were excluded during extraction, and its appearance was apparently correlated with loss of a fraction of cellular 23s rRNA. These results are similar to those of Howland and Ramus (9), who showed that approximately half of the 23s rRNA of the blue-green alga *Phormidium persicinium* may be converted to 0.88M material upon heating. The failure of either group to obtain complete conversion of the remaining intact 23s rRNA to discrete lower-molecular-weight fragments by heating or by exclusion of magnesium ions is consistent with data presented here (Table 1), which indicate that that fraction of *A. nidulans* 23s rRNA which remains intact through extraction and analysis in EDTA is not further converted to 0.88M RNA upon subsequent heat denaturation.

Szalay et al. observed that unlabeled RNA from stationary-phase cultures, or from cultures deprived of light, contains relatively more 0.88M RNA, and relatively less 23s rRNA, than does bulk RNA from exponentially growing cells (22; 0.17M RNA was not looked for in most experiments). They conclude that under suboptimal growth conditions the conformation of the total cellular ribosome population is altered in such a way as to render the rRNA more sensitive to a specific, magnesium-inhibitable, endonucleolytic attack during extraction. The results presented here make this interpretation appear unlikely. “Old” 23s rRNA is more extensively fragmented than “new” 23s rRNA contained within the same cell, regardless of growth conditions (Fig. 1). Lability of 23s rRNA is a function of time after transcription and is stimulated by, but does not require, light. The results of Szalay et al. with stationary-phase and unilluminated cultures can be reinterpreted as follows: in such cultures, RNA synthesis is reduced so that the bulk of unlabeled RNA which is present is in fact “older” than most of the rRNA found in exponentially growing cells, and it therefore appears more extensively fragmented when analyzed on polyacrylamide gels.

Strictly speaking, the present findings demonstrate only that *A. nidulans* ribosomes (or prefunctional ribonucleoprotein particles) are changed with time in such a way that 23s rRNA isolated from them in the absence of magnesium appears as two fragments on polyacrylamide gels. Although many possible time-dependent changes in either rRNA or protein structure might have this effect, the simplest would be specific in vivo endonucleolytic scission of the 23s rRNA occurring randomly after transcription and ribosome formation. As suggested by others (9, 22), the fragments resulting from cleavage (0.88M and 0.17M RNA) may remain noncovalently associated in the presence of magnesium ions. This proposed scheme is formally analogous to the scheme for production of 7s rRNA in mammalian cells (7, 18). Here, two species of rRNA (28s and 7s) are transcribed as parts of a single precursor and are separated by endonucleolytic cleavage at a later stage in ribosome maturation. The two fragments remain noncovalently associated on the ribosome throughout its functional life. Prematurational cleavages
such as this (and such as the conversions of precursor 23s and 16s rRNA to mature form) may be mechanistically related to the scission of A. nidulans 23s rRNA demonstrated here, and may differ only in that the former are known to precede and be required for ribosome function. The relationship between rRNA cleavage and ribosome function in A. nidulans is now under investigation.

The system described appears to be a good one with which to resolve at least some of the experimental and theoretical difficulties concerning rRNA "instability." The fragments produced by cleavage of A. nidulans 23s rRNA are distinct in size from each other and from 16s rRNA, and all are easily resolved on the same 2.8% polyacrylamide gel. The rate of cleavage is slow, and thus both precursor (23s rRNA) and product (fragments) can be prepared in good yield and high specific activity. Most promising is the fact that the rate of in vivo cleavage can be altered experimentally. It may be possible with A. nidulans to determine what rRNA instability means to the cell.

ACKNOWLEDGMENT

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LITERATURE CITED