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Polyadenylated RNA Isolated from the Archaebacterium *Halobacterium halobium*

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Polyadenylated [poly(A)$^+$] RNA has been isolated from the halophilic archaebacterium *Halobacterium halobium* by binding, at 4°C, to oligo(dT)-cellulose. *H. halobium* contains approximately 12 times more poly(A) per unit of RNA than does the methanogenic archaebacterium *Methanococcus vannielii*. The 3′ poly(A) tracts in poly(A)$^+$ RNA molecules are approximately twice as long (average length of 20 nucleotides) in *H. halobium* as in *M. vannielii*. In both archaebacterial species, poly(A)$^+$ RNAs are unstable.

Polyadenylation is an important structural feature of mRNAs in both eucaryotes and eubacteria. The poly(A) tracts, as well as the RNA molecules of which they are a part, are however quite different in the two groups. Eubacterial polyadenylated [poly(A)$^+$] RNAs exhibit a wide range of sizes and usually have only short poly(A) tracts (3–6, 9). They are unstable and therefore constitute only a small percentage of the total cellular RNA (3–5, 9, 11). We recently described the isolation and characterization of poly(A)$^+$ RNA molecules from the methanogenic archaebacterium *Methanococcus vannielii* (1) and found that they share the eubacterial properties of short 3′ poly(A) tracts, short half-lives and a broad size distribution. During our study of the methanogenic archaebacterium, somewhat conflicting results concerning polyadenylation of RNA in the halophilic archaebacterium *Halobacterium halobium* were published. The mRNA encoding bacterio-opsin in *H. halobium* does not appear to be poly(A)$^+$ (2), and yet a preliminary report was published describing very long, eucaryotic-like poly(A) tracts in RNA molecules isolated from *H. halobium* and from the thermoacidophilic archaebacterial genera *Thermoplasma* and *Sulfolobus* (10). We have now extended our studies to investigate *H. halobium* and report here that poly(A) sequences are present in *H. halobium* RNAs, but that the poly(A) tracts, although present in relatively large amounts, are much shorter than those normally found in eucaryotic mRNAs.

Poly(A)$^+$ RNA was isolated and quantitated from [^{3}H]uridine-labeled cultures of *H. halobium* by binding to oligo(dT)-cellulose at 4°C as previously described (1). Cultures were grown in rich *Halobacterium* medium (8) at 37°C with shaking and labeled as indicated (Table 1) by the addition of [^{3}H]uridine (10 μCi/ml). Of the radioactivity in labeled RNA isolated from cultures pulse-labeled with [^{3}H]uridine for 1/100 of a generation time, 11% was found to be in poly(A)$^+$ RNA (Table 1). Only 3.4% of the radioactively labeled RNA from cultures allowed to incorporate [^{3}H]uridine for several generations contained poly(A) sequences, indicating that the majority of the poly(A)$^+$ RNA molecules are not stable and are subject to turnover. This value (3.4%) for the steady-state percentage of RNA which contains poly(A) in *H. halobium* is, however, much higher than the values found for this parameter in *M. vannielii* (0.55%) and in *Escherichia coli* (0.68%). The half-life of *H. halobium* poly(A)$^+$ RNA was determined in pulse-chase experiments. Exponentially growing cultures were pulse-labeled with 10 μCi of [^{3}H]uridine per ml for 3 min, a 10,000-fold excess of unlabeled uridine was added, and the amount of poly(A)$^+$ RNA was determined at 1, 2.5, 5, 10, 20, 30, 45, and 60 min after the labeling period. The results obtained showed that the average half-life of poly(A)$^+$ RNA molecules in *H. halobium* at 37°C was 4.6 min, which is equivalent to 1/60 of a generation time.

The size distribution of radioactively labeled poly(A)$^+$ RNA from *H. halobium* was determined by electrophoresis of denatured RNA through agarose gels, followed by fractionation of the gel and scintillation counting of each gel slice. Poly(A)$^+$ RNA molecules were found to range from 0.5 to 3.0 kilobases, averaging 1.1 kilobase in length (Fig. 1). This size range is consistent with the presence of both monocistronic and polycistronic mRNAs and is similar to the range of sizes of mRNAs found in *M. vannielii* and in several eubacterial species (1, 5, 9, 11).

The amount of poly(A), as a fraction of the total cellular

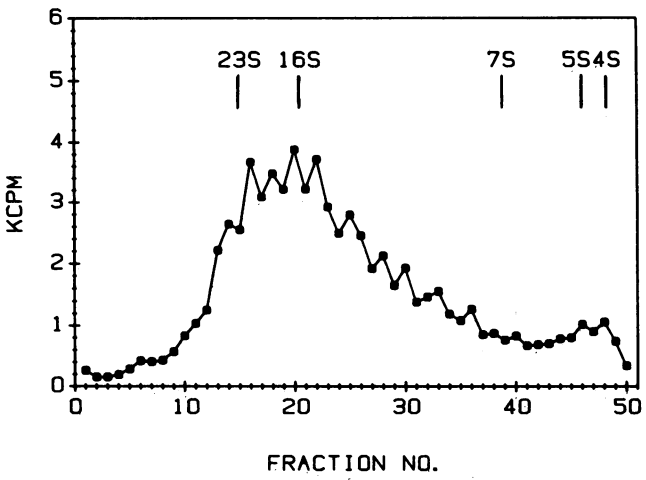

FIG. 1. Electrophoretic profile of *H. halobium* [^{3}H]uridine-labeled poly(A)$^+$ RNA. Poly(A)$^+$ RNA molecules, isolated from cells labeled for 0.01 generations, were denatured by glyoxylation and separated by electrophoresis through a 1.8% agarose gel. The locations of stable RNAs were determined by examination of adjacent lanes of the gel containing total RNA after staining with acridine orange. The gel in the appropriate lane was sliced into fractions, and the radioactivity in each fraction was determined.

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TABLE 1. Properties of *H. halobium* poly(A)$^+$ RNA and comparison with poly(A)$^+$ RNAs from *M. vannielii* and *E. coli*[a]

Organism	% Poly(A)$^+$ RNA after labeling[b]		Half-life (generations)	Poly(A)$^+$ RNA size (kilobases)		pg of poly(A)/ μg of RNA	Poly(A) length (bases)	
	Pulse	Extended		Range	Mean		Range	Mean
H. halobium	11.0	3.4	1/60	0.5–3.0	1.1	900	12–40	20
M. vannielii	15.9	0.55	1/24	0.7–2.8	1.2	69	6–30	12
E. coli	20.9	0.68	1/20	0.7–3.0	1.2	65	14–35	23

[a] Details of molecules from *M. vannielii* and *E. coli* are taken from reference 1. The procedures used to isolate and characterize the *H. halobium* poly(A)$^+$ RNA molecules are described in the text and in reference 1.

[b] Pulse, Labeled for 0.01 generation; extended, labeled for 3 generations. Percentage is for poly(A)$^+$ RNA compared with total radioactively labeled RNA.

RNA in *H. halobium*, was determined by measuring the amount of [^{3}H]poly(U) protected from RNase A and DNase I digestion by prehybridization with unlabeled *H. halobium* RNA. *H. halobium* was found to contain 900 pg of poly(A) per μg of total RNA, a fraction higher than the corresponding values for *M. vannielii* (69 pg/μg) and *E. coli* (65 pg/μg), but more than fivefold lower than the value obtained from yeast (5,000 pg/μg [1]). From the values obtained for (i) the steady-state percentage of total RNA which is poly(A)$^+$ RNA (3.4%), (ii) the amount of total RNA which is poly(A) sequences (900 pg/μg, or 0.09%), and (iii) the average length of poly(A)$^+$ RNA molecules (1,100 bases), it is possible to calculate the average length of poly(A) tracts in poly(A)$^+$ RNA molecules. Since poly(A) sequences are 0.09% and poly(A)$^+$ RNA is 3.4% of total RNA, poly(A) sequences must constitute 2.6% (0.09 ÷ 3.4) of the length of an average poly(A)$^+$ RNA molecule (1,100 bases); i.e., an average poly(A) tract must, by this calculation, be 29 bases in length.

The actual lengths of the 3′ poly(A) tracts were determined directly by RNase A and RNase T_1 digestion of poly(A)$^+$ RNA labeled at the 3′ terminus with ^{32}P, followed by electrophoresis of the products through sequencing gels and autoradiography (Fig. 2). *H. halobium* poly(A)$^+$ RNA was found to contain 3′ poly(A) tracts ranging in length from 12 to 40 bases, with an average length of 20 bases. This size is approximately twice the length of the poly(A) tracts found in *M. vannielii*, but is similar to the average length for poly(A) tracts reported from several eubacterial species, including *E. coli* (1, 3, 9, 11).

The discrepancy between the results reported here and the results reported by Oshima et al. (10) for the length of *H. halobium* poly(A) tracts may be a reflection of the fact that the method of RNA extraction used by Oshima et al. is not one which efficiently yields poly(A)$^+$ RNA from eubacteria and may, in fact, select against RNA molecules containing short poly(A) tracts (4). A very small percentage of the poly(A)$^+$ RNA molecules in *H. halobium* may contain the long (4S) poly(A) tracts described by Oshima et al.; however, the average length of poly(A) sequences in the poly(A)$^+$ RNA population in *H. halobium* is relatively short. The results described here indicate that the poly(A)$^+$ RNA molecules in *H. halobium* are essentially eubacterial in nature. Although we assume that the poly(A)$^+$ molecules

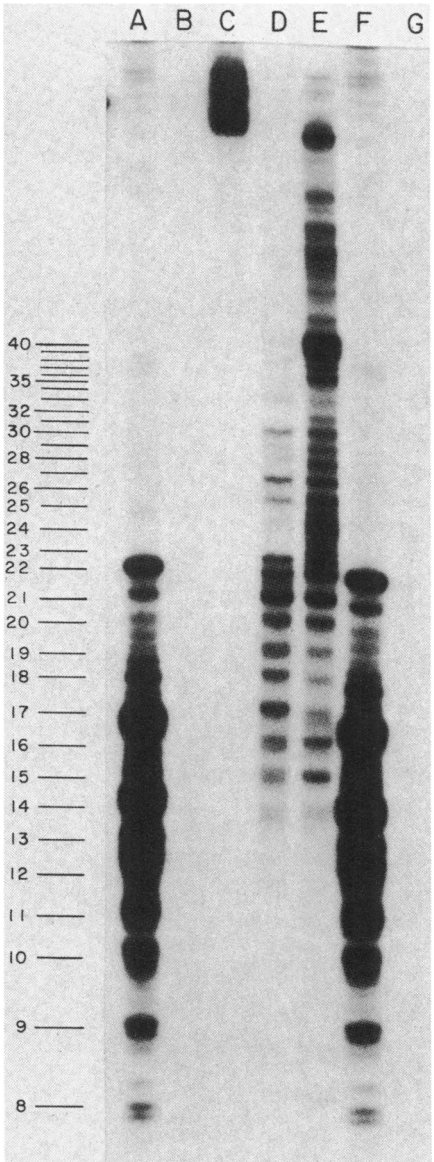

FIG. 2. Electrophoretic separation of 3′-end-labeled poly(A) tracts. *H. halobium* poly(A)$^+$ RNA molecules were 3′ end labeled with RNA ligase and [5′-^{32}P]cytidine-3′,5′-bisphosphate and then digested with RNase A and RNase T_1. Digestion-resistant oligomers were separated by electrophoresis through a DNA sequencing gel. The locations of molecular weight standards from a known DNA sequence, separated in adjacent tracks of the same gel, are shown on the left. Samples in the gel lanes are as follows: lanes A and F, 5′-end-labeled pBR322-*Msp*I molecular weight markers; lane B, KOH-digested poly(A)$^+$ RNA; lane C, untreated poly(A)$^+$ RNA; lane D, poly(A)$^+$ RNA digested with both RNase A and RNase T_1; lane E, the same as for lane D, except that a 1:125 dilution of the RNases was used; lane G, [5′-^{32}P]cytidine-3′,5′-bisphosphate, which migrated completely through the gel.

described in this report are mRNAs, this assumption remains to be proven. The presence of unusual RNAs in *H. halobium* has already been documented (7); we therefore should not be too conservative in our expectation of novelties in the molecular biology of archaebacteria.

LITERATURE CITED

1. **Brown, J. W., and J. N. Reeve.** 1985. Polyadenylated, noncapped RNA from the archaebacterium *Methanococcus vannielii*. J. Bacteriol. **162**:909–917.
2. **Chang, S. H., A. Majumdar, R. Dunn, O. Makabe, U. L. RajBhandary, H. G. Korana, E. Ohtsuka, T. Tanaka, Y. U. Taniyama, and M. Ikehara.** 1981. Bacteriorhodopsin: partial sequence of mRNA provides amino acid sequence in the precursor region. Proc. Natl. Acad. Sci. USA **78**:3398–3402.
3. **Crouch, D. H., J. D. Ownby, and N. G. Carr.** 1983. Polyadenylated RNA in two filamentous cyanobacteria. J. Bacteriol. **156**:979–982.
4. **Gopalakrisna, Y., D. Langley, and N. Sarkar.** 1981. Detection of high levels of polyadenylate-containing RNA in bacteria by the use of a single-step RNA isolation procedure. Nucleic Acids Res. **9**:3345–3354.
5. **Hussain, I., N. Tsukagoshi, and S. Udaka.** 1982. Characterization of polyadenylated RNA in a protein-producing bacterium, *Bacillus brevis* 47. J. Bacteriol. **151**:1162–1170.
6. **Majumdar, P. K., and B. A. McFadden** 1984. Polyadenylated mRNA from the photosynthetic procaryote *Rhodospirillum rubrum*. J. Bacteriol. **157**:795–801.
7. **Moritz, A., B. Laskat-Buttgereit, H. J. Gross, and W. Goebel.** 1985. Common structural features in the genes for two stable RNAs from *Halobacterium halobium*. Nucleic Acids Res. **13**:31–43.
8. **Oesterhelt, D., and W. Stoeckenius.** 1974. Isolation of the cell membrane of *Halobacterium halobium* and its fractionation into red and purple membrane. Methods Enzymol. **31**:667–678.
9. **Ohta, N., M. Sanders, and A. Newton.** 1978. Characterization of unstable poly(A)-RNA in *Caulobacter crescentus*. Biochem. Biophys. Acta **517**:65–75.
10. **Oshima, T., M. Obha, and T. Wakagi.** 1984. Some biochemical properties of an acido-thermophilic archaebacterium, *Sulfolobus acidocaldarius*. Origins Life **14**:665–669.
11. **Schultz, G. A., G. Chaconas, and R. L. Moore.** 1978. Polyadenylic acid sequences in the RNA of *Hyphomicrobium*. J. Bacteriol. **133**:569–575.