[Regular paper]

Electrophoretic characterization of low molecular weight RNAs from prokaryotic and eukaryotic cells

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SUMMARY

Low molecular weight RNAs (small RNAs=sRNAs) from prokaryotic and eukaryotic cells were analyzed parallelly by electrophoresis in an agarose-polyacrylamide composite gel and a 7 M urea-10% polyacrylamide gel.

Induced by chloromycetin, three major sRNAs (approx. 190, 240 and 320 nucleotides long) were found to be synthesized during germination of *Bacillus subtilis* spores. These RNAs were distinct from the commonly occurring 4S, 4.5S and 5S RNAs.

The U-series small nuclear RNAs (U-snRNAs) from KB cell nuclei were also separated by gel electrophoresis. The number of U-snRNA molecules in the cell nucleus was calculated using a reported value for U1 RNA as the standard. When the number of U1 RNA was 1×10^6 molecules in a cell, those of U2, U4, U5 and U6 RNAs were estimated to be 2.7×10^5 , 1.3×10^5 , 5.4×10^4 and 1.3×10^5 molecules, respectively.

In F9 cells, a mouse teratocarcinoma cell line, U1a and U1b RNA were observed as to U1 RNA.

In each gel electrophoresis, the logarithms of the (assumed) molecular weights of sRNAs were inversely proportional to the mobility.

Key words : agarose-polyacrylamide composite gel, urea-polyacrylamide gel, low molecular weight RNA

原核細胞および真核細胞における低分子量 RNA 分子種の尿素-ポリアクリルアミドゲル電気泳動法による分画
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(Received October 2, 1985, Accepted January 14, 1986)

Introduction

Cells of the prokaryote, *Escherichia coli*, have been reported to contain certain low molecular weight RNAs (small RNAs=sRNAs), other than 4S tRNA and 5S rRNA¹⁻⁴). Most eukaryotic cells also contain numbers of small nuclear and cytoplasmic RNAs (snRNAs and scRNAs)⁵⁻⁸). In the analysis of gene expression, one area of current interest is the role played by these small RNA molecules⁸⁻¹²).

Our effort has been directed to isolation and characterization of sRNAs. Electrophoresis in polyacrylamide gel containing urea is very useful for analyzing these small RNAs. The present paper reports the electrophoretic characterization of the low molecular weight RNAs, *Bacillus subtilis*, a prokaryote and of certain eukaryotic cells.

Materials and methods

1) Bacterial strains, media, growth conditions and isotope labeling of RNA

Bacillus subtilis 168 LMH (Leu⁻Met⁻His⁻) was used throughout this study for prokaryotic RNA

analysis. The media, growth conditions and isotope labeling of RNA were the same as described previously¹³.

2) Eukaryotic cells, culture conditions and isotope labeling of RNA

Human KB cells were grown in Joklik's modified minimum essential medium supplemented with 5% horse serum and labeled for 16 h with [5,6] ⁻³H uridine (10 μ Ci/ml) at 2×10^5 cells/ml.

Mouse F9 embryonal carcinoma cells were grown in Dulbecco's modified Eagle medium containing 10% fetal calf serum and labeled for 14 h with ³²P (18 μ Ci/ml) at 1.6×10^5 cells/ml.

3) RNA sample preparation

³H-RNA of the bacteria was prepared as described previously¹³. Eukaryotic RNA was extracted with saturated phenol in the presence of SDS at room temperature. The RNA was precipitated with ethanol.

4) Polyacrylamide gel electrophoresis (PAGE) of RNA

RNAs were analysed by electrophoresis either in a 0.5% agarose-2% polyacrylamide composite gel¹⁴) or in a 10% polyacrylamide gel containing 7 M

Fig.1. 0.5% agarose-2% polyacrylamide composite gel electrophoresis of ³H-RNA synthesized during the vegetative log-phase growth stage of *Bacillus subtilis* 168 LMH (a). The bands were scanned at 550nm (b). The relation between the molecular weights of RNA species and their mobilities is demonstrated (c).

Fig.2. 10% PAGE in the presence of 7 M urea of ^3H -RNA pulse labeled for 1 min during the first 10 min of germination of *Bacillus subtilis* spores (a). Scanning of the bands (b) and the relation between molecular weights and mobility (c).

urea¹⁵⁾, followed by fluorography¹⁶⁾ when necessary.

Results and discussion

The RNA profile in the agarose-polyacrylamide composite gel of transcripts synthesized during the vegetative log-phase growth of *Bacillus subtilis* is shown in Fig. 1 as a standard pattern. The dominant RNA bands are of 16S and 23S rRNAs and 4 to 5 S RNA species, using radiolabeled whole cell extract as the starting material (Fig. 1a), in agreement with the report of Peacock and Dingman¹⁴⁾. These RNA bands were scanned at 550 nm (Fig. 1b). The ratio of 23S rRNA/16 S rRNA/4~5 S RNA was estimated to be about 23/19/7 from the data shown in Fig. 1 b. The logarithm of assumed molecular weight of each RNA¹⁷⁾ was inversely proportional to the mobility in this composite gel (Fig. 1 c). For better resolution of the 4~10 S sRNA species other than 4S tRNA and 5S rRNA in *Bacillus subtilis*, electrophoresis was carried out in a 10% polyacrylamide gel containing 7M urea, in an attempt to separate the RNA products transcribed

Fig.3. 7 M urea-10% PAGE of ^3H -RNA. CM (100 $\mu\text{g}/\text{ml}$) was given at time 0 and RNAs were pulse labeled (1 min) at 15 min during germination of *Bacillus subtilis* (a). Scanning of the bands (b).

at an early stage, i. e., during the first 10 min of the germination of *Bacillus subtilis* spores. The smaller RNA species synthesized during this period were 4 S, 4.5 S and 5S RNAs and four minor species which were designated as LMW I, II, III, IV. The sizes were estimated as 190 nucleotides for LMW I, 240 for III and 320 for IV, respectively, when their mobilities were compared with those of human KB RNAs (Fig. 2 c).

To characterize the RNAs synthesized early in germination, the effects of chloromycetin (CM :

100 $\mu\text{g/ml}$), a potent inhibitor of bacterial translation, on synthesis of the RNA species were examined, since CM-induced changes in the synthesis of some RNA species in *Escherichia coli* have been reported^{18,19}. It is evident from Fig. 3 that the synthesis of three species, LMW I, III and IV, is stimulated by CM. These RNAs were produced in the same amounts as that of 5S rRNA in the presence of CM (Fig. 3 b). Unfortunately, we can offer no explanation at present for the physiological functions of these RNAs. Their characterization and the

Fig.4. 7 M urea-10% PAGE of KB cell nuclear RNA (a2) and cytoplasmic RNA species (a1) labeled with ^3H -uridine for 16h. (b) and (c) are the same as in Fig.2.

analysis of their functions, as well as studies on their induction by CM are now in progress.

Next, gel electrophoretic characterizations of low molecular weight RNAs in certain eukaryotic cells were carried out.

Fig. 4 a shows the patterns of small cytoplasmic and small nuclear RNAs(sn RNAs) from cultured human KB cells electrophresed in the 10% polyacrylamide gel containing 7M urea. The logarithms of the nucleotide lengths of the RNAs between U6 and 7S RNAs were inversely proportional to their mobilities in this gel (Fig. 4 c) .About 1×10^6 molecules of U1 RNA, an abundant source of snRNA, are considered to be present in a human cell and about 1×10^4 to 5×10^5 molecules each in the case of other U-snRNAs^{20,21)}. The number of other

U-snRNA molecules in a human KB cell was calculated, using the value for U1 RNA in a human cell reported and the relative amounts of U-snRNAs in the gel (Fig. 4 b) . The results obtained are shown in Table 1.

snRNAs are thought to be functional when associated with the protein moiety forming the socalled small nuclear ribonucleoprotein particles (snRNPs).Some suggestions have been made as to the functions of U-snRNPs⁸⁻¹¹⁾. Clarification of the structure and functions of snRNPs should prove to be important.

F9 cell is one of several cloned lines of mouse embryonal carcinoma stem cells that, under normal tissue culture conditions, does not give rise to differentiated cells^{22,23)}. The RNA profile of F9 cellular small RNAs on the gel indicated that the logarithms of the nucleotide lengths of RNAs between U6 and 7S RNA were inversely proportional to their mobilities (Fig. 5 c)

Of the species examined, chicken²⁴⁾, human and insect¹⁰⁾ cells contain only one species of U1 RNA. Recently, Forbes et al. reported that *Xenopus laevis* cells contain multiple U1 RNA species, some of

Table 1. Estimated numbers of U snRNA molecules in a human KB cell.

U snRNA	the number of molecules
U1	1.0×10^6
U2	2.7×10^5
U4	1.3×10^5
U5	5.4×10^4
U6	1.3×10^5

Fig.5. 7 M urea-10% PAGE of mouse teratocarcinoma F9 cell RNA species labeled with ³²P for 14 h. (b) and (c) are the same as in Fig.2.

which are transcribed specifically in embryos²⁵⁾. In mouse and rat cells, two electrophoretically distinguishable U1 RNAs (U1a and U1b RNA) have been found^{26,27)}.

We also found two species of U1 RNAs in F9 cells (Fig.5 a and b). Two species of U1 snRNA and/or snRNP have been observed in Ehrlich ascites cells and Friend erythroleukemia cells^{26,27)}. The proportion of U1a-snRNP to U1b-snRNP has previously been found to be high in the F9 cells²⁸⁾. The F9 cell line may possibly be useful for studying the mechanism(s) regulating differential gene expression of RAN or protein components in U1 snRNP(s) and/or the differential assembly of U1-snRNP(s) during developmental process.

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要 旨

原核細胞(枯草菌)と真核細胞(ヒト KB およびマウステラトカルシノーマ F9細胞)の低分子量 RNA をはじめて平行してアガロース-ポリアクリルアミド複合ゲルおよび尿素-ポリアクリルアミドゲル電気泳動法を用いて解析した。

まず、枯草菌では発芽初期に 4s tRNA や 5s rRNA とは異なる、少なくとも 4 つの低分子量 RNA, LMW I, II, III, IV が検出された。また、LMW I, III, IV はその合成がクロロマイセチン投与によって促進される RNA 分子種であり、さらにそのヌクレオチドの長さはそれぞれ 190, 240, 320 と推定された。

ヒト KB 細胞核では U1, U2, U4, U5 および U6 snRNA が解析され、スキヤニングの結果より U2, U4, U5, U6 snRNA の細胞当たりのコピー数が計算された。

一方 F9 細胞では従来のマウス細胞を用いたいくつかの報告と一致して U1 RNA に関して 2 種類 (U1a と U1b) が検出され他に 7S, U2, U4, U6 等の snRNA が検出された。

報告中で示したすべての RNA-ゲル電気泳動系において、解析された RNA の分子量(またはヌクレオチド長)の対数とその移動度に正しく逆比例している事が確認された。