Cell-free Transcription of Methanogen Genes

Besides the DNA-dependent RNA polymerase at least two transcription factors are required for accurated initiation of transcription in archaea (1). We describe a one step procedure allowing the purification of all protein-components of an *in vitro* transcription system of *Methanococcus thermolithotrophicus*.

BASIC PROTOCOL

Materials

TK buffer
Transcription mixture
11 mMATP
11 mM GTP
11 mM CTP
0.07 mM UTP
4 µCi α-^{32}P-UTP
0.6 M Na-acetate, pH 7.0
Phenol
Chloroform
Deonized formamide with 0.01 percent xylene cyanol and bromphenol blue
TBE buffer

Purification of the extract

Unless otherwise indicated all procedures are performed at room temperature under anaerobic conditions.

1. Suspend 2 g cells in 8 ml TK buffer using a Potter Elvehjem homogenizer.

2. Lyse the cells using a French pressure cell at 140 MPa and clear the extract by centrifugation in a Beckman 50 Ti rotor at 39,000 rpm for 1 h at 0°C.

3. Apply the supernatant to a 8 ml phosphocellulose-column (1 x 10 cm) and wash with three column-volumes of TK buffer.

4. Elute the bound protein in steps with TK buffer containing 0.35 and 0.8 M KCl.
   The RNA polymerase and one transcription factor are eluted at 0.35 M KCl, the second transcription factor at 0.8 M KCl.

Assay for cell-free transcription and analysis of RNA products

1. Perform transcription reactions in a 100 µl of transcription mixture. Add template DNA to a final concentration of 30 µg/ml.

2. Preincubate the RNA polymerase and transcription factor fractions (20 µl each) with the template for 5 min at 55°C.

3. Begin the reaction by the addition of 11 mM of each ATP, GTP, CTP; 0.07 mM UTP and 4 µCi α-^{32}P-UTP. Incubate 30 min at 55°C.

4. Stop RNA synthesis by the addition of 100 µl 0.6 M Na-acetate, pH 7.0 followed immediately by 100 µl phenol and 100 µl chloroform.

5. Extract the aqueous phase with 1 volume chloroform.

6. Precipiate the nucleic acids with 3 volumes of ethanol.

7. Suspend the pellet in 4 µl deonized formamide containing 0.01% xylene cyanol and bromphenol blue. Denature the RNA for 2 min at 90°C.

8. Apply the sample to a 6-8% polyacrylamide urea gel (1 mm) in 1X TBE buffer. Electrophorese at 30 mA for 2 h.

9. Visualize the labelled RNA by autoradiography after an exposure time of 20 min at -80°C.

REAGENTS AND SOLUTIONS

TK buffer
50 mM Tris HCl, pH 8.0
100 mM KCl
20% Glycerol (v/v)

Transcription mixture
20 mM Tris-HCl, pH 8.0
10 mM $MgCl_2$
50 mM KCl
0.1 mM EDTA.

COMMENTARY

This extract directs faithful cell-free transcription of both tRNA and protein-encoding genes (2) of *Methanococcus* from supercoiled templates. Genes of *Methanothermus fervidus* are also specifically transcribed (3) suggesting that this system is suitable to study the transcription of cloned genes of various genera of methanogens.

The purified extracts stored in portions at -80°C are stable for several months

References

1. Frey, G., Thomm, M., Brudigam, B., Gohl, H. P. and Hausner, W. 1990. *Nucl. Acids Res.* 18:1361-1367

2. Gohl, H. P., Hausner, W. and Thomm, M. 1990. manuscript in preparation

3. Thomm, M., Reeve, J., Sandmann, K. and Frey, G., unpublished data

Contributors

Michael Thomm and Gerhard Frey, Lehrstuhl fur Mikrobiologie, Universitat Regensburg, D-8400 Regensburg, Federal Republic of Germany