Available online at www.sciencedirect.com
ScienceDirect
ELSEVIER
Plasmid 57 (2007) 265–274
PLASMID
www.elsevier.com/locate/yplas

Adenosine monophosphate-induced amplification of ColE1 plasmid DNA in *Escherichia coli*

Zhijun Wang [a,b], Li Xiang [a], Junjie Shao [a], Grzegorz Węgrzyn [c,d,*]

[a] *Key Laboratory of Medical Molecular Virology, Shanghai Medical College, Fudan University, 200032 Shanghai, PR China*
[b] *Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853, USA*
[c] *Department of Molecular Biology, University of Gdańsk, Kładki 24, 80-822 Gdańsk, Poland*
[d] *Department of Genetics and Marine Biotechnology, Institute of Oceanology, Polish Academy of Sciences, Św. Wojciecha 5, 81-347 Gdynia, Poland*

Received 20 April 2006, revised 4 October 2006
Available online 28 November 2006
Communicated by Dhruba Chattoraj

Abstract

ColE1 plasmid copy number was analyzed in relaxed (*relA*) and stringent (*relA*$^+$) *Escherichia coli* cells after supplementation of culture media with adenosine monophosphate (AMP). When a relaxed *E. coli* strain bearing ColE1 plasmid was cultured in LB medium for 18 h and induced with AMP for 4 h, the plasmid DNA yield was significantly increased, from 2.6 to 16.4 mg l^{-1}. However no AMP-induced amplification of ColE1 plasmid DNA was observed in the stringent host. Some plasmid amplification was observed in *relA* mutant cultures in the presence of adenosine, while adenine, ADP, ATP, ribose, potassium pyrophosphate and sodium phosphate caused a minor, if any, increase in ColE1 copy number. A mechanism for amplification of ColE1 plasmid DNA with AMP in *relA* mutant bacteria is suggested, in which AMP interferes with the aminoacylation of tRNAs, increases the abundance of uncharged tRNAs, and uncharged tRNAs promote plasmid DNA replication. According to this proposal, in *relA*$^+$ cells, the AMP induction could not increase ColE1 plasmid copy number because of lower abundance of uncharged tRNAs. Our results suggest that the induction with AMP can be used as an effective method of amplification of ColE1 plasmid DNA in relaxed strains of *E. coli*.
© 2006 Elsevier Inc. All rights reserved.

Keywords: ColE1 plasmid; DNA replication; Plasmid amplification; *Escherichia coli relA* mutant

1. Introduction

ColE1 plasmid requires only proteins encoded by its host, *Escherichia coli*, for replication (Donoghue and Sharp, 1978). The regulatory molecules involved in the control of ColE1 replication include RNA I, RNA II, Rom, DNA polymerase I (Pol I) and RNase H (Kues and Stahl, 1989; Merlin and Polisky, 1995). RNA II forms a hybrid with the replication origin, and after its cleavage by RNase H the released 3′OH end is elongated by Pol I (Itoh and Tomizawa, 1980). RNA I is an antisense RNA for RNA II. The binding of RNA I to

* Corresponding author. Fax: +48 58 301 0072.
E-mail address: wegrzyn@biotech.univ.gda.pl (G. Węgrzyn).

0147-619X/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.plasmid.2006.10.002

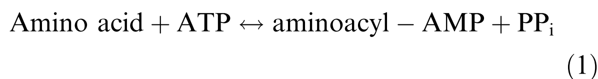
RNA II inhibits an interaction of the latter with DNA template (Itoh and Tomizawa, 1980). A plasmid-encoded Rom protein is another factor regulating ColE1 plasmid DNA replication. This protein can stimulate the binding of RNA I to RNA II (Kues and Stahl, 1989; Lin-Chao et al., 1992).

ColE1 plasmid and its derivatives and relatives are extensively used as vectors for gene cloning, gene therapy and DNA vaccine studies. Thus, downstream processing of plasmid DNA acquired a considerable interest (Wang et al., 2001, 2002a,c). It is estimated that the market of plasmid DNA could exceed 45 billion USD in 2010 (Prazeres et al., 1999), hence studies on ColE1 plasmid replication and amplification are not only important for answering basic scientific questions but also have economic prospects (Grabherr and Bayer, 2002; Wang et al., 2004).

Amplification of ColE1-derived plasmids has been observed in response to chloramphenicol (Clewell, 1972), isopropyl-β-D-thiogalacto-pyranoside (IPTG)-induction of a cloned gene (Teich et al., 1998), and amino acid starvation (Hanschke and Hecker, 1986; Hecker et al., 1983, 1986a,b; Wang et al., 2002b; Wegrzyn, 1999). In all these systems, a significant increase in plasmid copy number was observed after induction. The rate of amplification and the final plasmid content per cell were dependent on the actual system used.

During amino acid starvation, uncharged tRNAs were proposed to be crucial factors in stimulation of replication of ColE1-like plasmid DNA in *relA* hosts (Wang et al., 2002b; Wegrzyn, 1999; Wrobel and Wegrzyn, 1998). Therefore, we aimed to use a chemical method to induce an appearance of uncharged tRNA and to increase the ColE1 plasmid DNA copy number in *E. coli*.

The attachment of an amino acid to a tRNA molecule can be described as follows:



$$\text{Amino acid} + \text{ATP} \leftrightarrow \text{aminoacyl} - \text{AMP} + \text{PP}_i \quad (1)$$

$$\text{Aminoacyl} - \text{AMP} + \text{tRNA} \leftrightarrow \text{aminoacyl} - \text{tRNA} + \text{AMP} \quad (2)$$

An oxygen atom of the amino acid α-carboxyl group attacks the phosphorus of the initial phosphate of ATP (Eq. 1). Then, the 2′ or 3′ hydroxyl group of the terminal adenosine of tRNA attacks the amino acid carbonyl carbon atom (Eq. 2). Each aminoacyl-tRNA synthetase (aaRS) recognizes specific amino acid and tRNA.

According to Eq. (2), one could suppose that AMP might be used to increase uncharged tRNA levels, and thus, to increase ColE1 plasmid DNA copy number in *E. coli*. In fact, Airas (2006) has demonstrated that an addition of 0.5 mM AMP can significantly inhibit tRNA aminoacylation *in vitro*. In biotechnology, AMP can potentially be used for large-scale production; for example, AMP induction was found to be an effective method to induce the synthesis of recombinant proteins in the cultivation of mammalian cells (Carvalhal et al., 2003; Luo et al., 2005). Therefore, we aimed to test whether AMP can be used to increase ColE1 plasmid DNA copy number.

2. Materials and methods

2.1. Bacterial strains, plasmids and cultivation conditions

Escherichia coli K-12 strain CP78 (*leu*, *arg*, *thr*, *his*, *thi*), referred to as $relA^{+}$, and its isogenic *relA2* derivative, CP79 (Fiil and Friesen, 1968), were employed. ColE1 plasmid (GenBank Accession No: NC_001371) was used. ColE1M plasmid is a ColE1 derivative, lacking the *rom* gene. For construction of ColE1M, PCR amplification with *Pfx* DNA polymerase, 5′-phosphorylated primers (5′-TGA TGC CTC CGT GTA AGG GGG ATT TCT GTT CAT GGG and 5′-CAC ATG CAG CTC CCG GAG ACG GTC ACA GCT TGT CTG) and ColE1 DNA template, was performed. The PCR product, consisting of a linear ColE1 sequence devoid of *rom*, was used for a ligation reaction with T4 DNA ligase, to construct plasmid ColE1M.

Cultivations were performed in a 5 l BIOSTAT B-DCU fermenter (Sartorius BBI Systems Inc., Melsungen) at controlled temperature (37 °C) and pH (7.0), with the cultivation volume of 500 ml. Following initial cultivation for 18 h, the medium was exchanged (or not) for the fresh one, with addition (or not) of chemical reagent(s), and cultivation was continued for another 4–6 h.

M9-glucose minimal medium supplemented with necessary amino acids (the medium was composed of: 12.8 g l^{-1} $Na_2HPO_4 \cdot 7H_2O$; 3.1 g l^{-1} KH_2PO_4; 0.5 g l^{-1} NaCl; 1.0 g l^{-1} NH_4Cl; 0.5 g l^{-1} $MgSO_4 \cdot 7H_2O$; 4.0 g l^{-1} glucose, and required amino acids: L-leucine, L-histidine, L-arginine, and L-threonine, 50 mg l^{-1} each) was used for amino acid starvation experiments. Amino acid starvation was achieved by removal of all amino acids from the medium. Briefly, bacterial culture was centrifuged, and the pellet was washed twice with an equal volume of 0.9% NaCl and resuspended in the M9-glucose minimal medium lacking amino acids.

Download English Version:

https://daneshyari.com/en/article/2824436

Download Persian Version:

https://daneshyari.com/article/2824436

Daneshyari.com