Gene, 151 (1994) 143–145
© 1994 Elsevier Science B.V. All rights reserved. 0378-1119/94/$07.00

GENE 08314

A small, high-copy-number vector suitable for both in vitro and in vivo gene expression

(Cloning; coupled transcription/translation system of Zubay; *Escherichia coli*; phospholipase A_2; adenylate kinase; chicken)

Baohua Huang, Zhengtao Shi and Ming-Daw Tsai

Ohio State Biochemistry Program and Department of Chemistry, The Ohio State University, Columbus, OH 43210, USA

Received by J.P. Richardson: 11 April 1994; Revised/Accepted: 22 June 1994; Received at publishers: 1 August 1994

SUMMARY

A 2433-bp, high-copy-number expression vector, pUK, was constructed from pUC19 and pKK223-3. The genes encoding chicken adenylate kinase (AK) and phospholipase A_2, cloned into pUK, were highly expressed in vitro using the coupled transcription/translation system of Zubay. High-level in vivo expression in *Escherichia coli* was also demonstrated for the *AK* gene cloned in this vector. The multiple cloning site (MCS) of pKK223-3 was preserved in pUK. In addition, three more sites in the MCS sequence, *Acc*I, *Bam*HI and *Sal*I, were made unique for the convenience of cloning.

INTRODUCTION

In vitro gene expression using the coupled transcription/translation system (Zubay, 1973; Pratt, 1984) requires the preparation of highly supercoiled plasmid DNA in large quantity. However, the widely used expression vectors with a strong promoter, such as the pKK series (Brosius et al., 1984; Amann, 1985) and pTrc99A (Amann, 1988) vectors, are relatively large in size (approx. 5 kb) and low in copy numbers. Large-size plasmid DNAs are less easy to purify in supercoiled form and the low-copy-number feature limits the yield of DNA. Thus, it is desirable to have an expression vector of small size and high copy number for in vitro gene expression.

Correspondence to: Dr. M.-D. Tsai, Department of Chemistry, Ohio State University, 120 W. 18th Avenue, Columbus, OH 43210-1173, USA. Tel. (1-614) 292-3080; Fax (1-614) 292-1532.

Abbreviations: AK, adenylate kinase; bp, base pair(s); Ap, ampicillin; IPTG, isopropyl-β-D-thiogalactopyranoside; kb, kilobase(s) or 1000 bp; MCS, multiple cloning site(s); nt, nucleotide(s); *ori*, origin of DNA replication; PAGE, polyacrylamide-gel electrophoresis; PLA_2, phospholipase A_2; SD, Shine-Dalgarno (sequence); SDS, sodium dodecyl sulfate.

SSDI 0378-1119(94)00525-7

EXPERIMENTAL AND DISCUSSION

(a) Construction of pUK

pUK was constructed as a hybrid vector consisting of the high-copy-number replicon and the Ap^R derived from pUC19 (Yanisch-Perron et al., 1985), along with the strong *tac* promoter, the SD sequence, the MCS and the *rrnB* transcription terminator derived from pKK223-3 (Brosius et al., 1984). As shown in Fig. 1, the pUC19 component was a 1811-bp *Aat*II-*Afl*III fragment that had been treated with T4 DNA polymerase to create blunt ends while the pKK223-3 segment was a 622-bp *Ssp*I fragment. *Ssp*I cuts pKK223-3 16 bp upstream from the −35 region of the *tac* promoter and at nt 4050, which is downstream from the *rrnB* terminator sequence. The two fragments were ligated to yield the plasmid pUK. The relative orientation of the two fragments was confirmed by mapping pUK by digestion with *Eco*RI and *Pvu*I. In addition to the unique sites at the MCS of pKK223-3, three more sites, *Acc*I, *Bam*HI and *Sal*I, were made unique by eliminating the duplicate sites present in pKK223-3 for these enzymes. This feature makes pUK a more versatile vector for cloning.

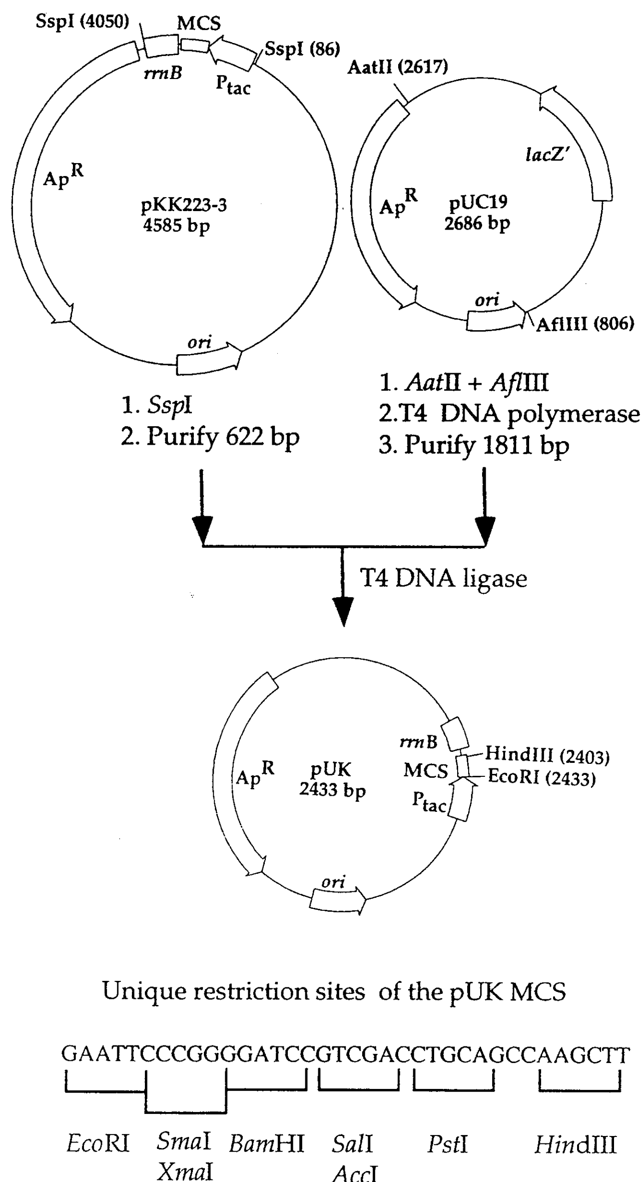

Fig. 1. Construction of pUK, a hybrid vector of pKK223-3 and pUC19. The part containing the *tac* promoter (P_{tac}), the SD sequence, MCS and a transcription terminator (*rrn*B) was from pKK223-3. The other part of pUK containing the Ap-resistance-encoding gene (Ap^R) and the replication elements (*ori*) was from pUC19. All the unique sites in MCS are shown.

(b) In vitro gene expression using the pUK vector

Two genes, encoding the bovine pancreatic phospholipase A_2 (PLA_2) (Deng et al., 1990) and the chicken muscle adenylate kinase (AK) (Tanizawa et al., 1987), which were previously expressed in vitro using pKK223-3 vector in our lab, (unpublished data), were used to test the pUK. The PLA_2 gene was subcloned into pUK between *Eco*RI and *Pst*I sites and the *AK* was subcloned into this vector between *Eco*RI and *Hin*dIII sites. In the in vitro expression experiments using the coupled transcription/translation system, both PLA_2 and AK genes were expressed when the reactions were primed with pUK containing the corresponding gene (Fig. 2). The expression levels were comparable to those directed by the pKK223-3 vector containing the corresponding genes as shown in Fig. 2. Furthermore, the small-size and high-copy-number features of pUK allow the isolation of highly supercoiled plasmid in very high yield (approx. 2.5 mg DNA/litre of culture). It was estimated that the pUK-based plasmids produce approx. 10-times more plasmid DNA than the corresponding pKK series vectors in the standard large-scale DNA preparation using the CsCl gradient method.

The pUK vector was also shown to direct in vivo gene expression in *E. coli.* When pUK containing *AK* gene was introduced into JM105 cells, AK was expressed at a high level shortly after induction by IPTG based on SDS-PAGE analysis (results not shown).

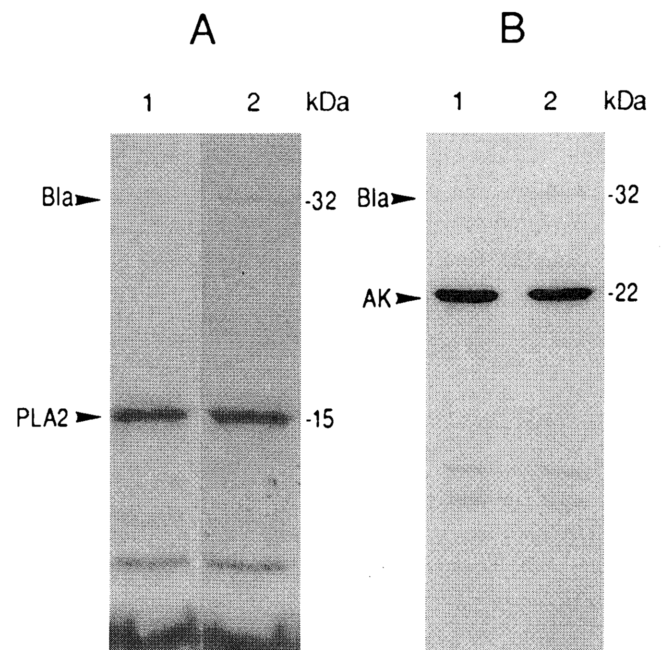

Fig. 2. Comparison of in vitro expressions of *AK* and PLA_2 genes cloned into pUK and pKK223-3. For a 30-μl reaction, plasmid DNA was mixed with 8.5 μl *E. coli* S-30 extract/7.5 μl low-molecular-weight mixture (containing the four NTP/phospho*enol*pyruvate/folinic acid/*E. coli* tRNAs/20 amino acids)/1 μl [^{35}S]methionine/1.5 μl Mg·acetate under the conditions described by Ellman et al. (1991). The reaction mixture was incubated at 37°C for 1 h on a rotary shaker (approx. 200 rpm). The samples were then centrifuged and the supernatant was analyzed by 0.1% SDS-15% PAGE (Laemmli, 1970). Electrophoresis was carried out at 20 mA for 4 h, and the gel was subjected to autoradiography. (**A**) In vitro synthesis of PLA_2. The in vitro expression reaction was primed with 3 μg plasmid DNA carrying the PLA_2 gene cloned into the pKK223-3 vector (lane 1) or the pUK vector (lane 2); 5 μl of the reaction mixture was loaded for each lane. (**B**) In vitro synthesis of AK. The in vitro expression reaction were primed with 3 μg plasmid pKK223-3 DNA carrying the *AK* gene (lane 1) or 1.8 μg of plasmid pUK DNA carrying the *AK* gene (lane 2). 1 μl of the reaction mixture was loaded for each lane. Notice that equal molar amounts of the two vector DNAs were used in lanes 1 and 2.

(c) Conclusions

The pUK vector has been constructed and demonstrated to be especially advantageous for the in vitro expression of genes using the coupled transcription/translation system which works best with high concentrations of highly supercoiled DNA. This vector has also been shown to be suitable for in vivo gene expression in *E. coli*. The presence of three additional unique sites at the MCS of pUK than that of pKK223-3 should make it more convenient for cloning genes into pUK.

ACKNOWLEDGEMENTS

This work was supported by NIH grants GM41788 and GM43268 to M.-D.T.

REFERENCES

Brosius, J. and Holy, A.: Regulation of ribosomal RNA promoter with a synthetic *lac* operator. Proc. Natl. Acad. Sci. USA 81 (1984) 6929–6933.

Amann, E. and Brosius, J.: 'ATG vectors' for regulated high-level expression of cloned genes in *Escherichia coli.* Gene 40 (1985) 183–190.

Amann, E., Ochs, B. and Abel, K.-J.: Tightly regulated *tac* promoter vectors useful for expression of unfused and fused proteins in *Escherichia coli.* Gene 69 (1988) 301–315.

Deng, T., Joseph, P.N. and Tsai, M.-D.: A novel expression vector for high-level synthesis and secretion of foreign proteins in *Escherichia coli.*: overproduction of bovine pancreatic phospholipase A_2. Gene 93 (1990) 229–234.

Ellman, J., Mendel, D., Anthony-Cahill, S., Noren, C.J. and Schultz, P.G.: Biosynthetic method for introducing unnatural amino acids site-specifically into proteins. Methods Enzymol. 202 (1991) 301–336.

Laemmli, U.K.: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227 (1970) 680–685.

Pratt, J.M.: Coupled transcription-translation in prokaryotic cell-free system. In: Hames, B.D. and Higgins, S.J. (Eds.), Transcrition and Translation: A Practical Approach. IRL Press, Oxford, 1984, pp. 179–209.

Tanizawa, Y., Kishi, F., Kaneko, T., and Nakazawa, A.J.: High level expression of chicken musle adenylate kinase in *Escherichia coli.* Biochem. J. 101 (1987) 1289–1296.

Yanisch-Perron, C., Vieira, J. and Messing, J.: Improved M13 phage cloning vectors and host strains: nucleotide sequence of the M13mp18 and pUC19 vectors. Gene 33 (1985) 103–109.

Zubay, G.: In vitro synthesis of protein in microbial systems. Annu. Rev. Genet. 7 (1973) 267–287.