Acta Biochimica Polonica

Vol. 42 No. 2/1995

275–280

QUARTERLY

Short Communication

Cloning and expression of the genes coding for tube associated proteins of bacteriophage T4

Józef Nieradko

Department of Microbiology, University of Gdańsk, Kładki 24, 80–822, Gdańsk, Poland

Received: 5 May, 1994; accepted: 3 March, 1995

Key words: bacteriophage T4, baseplate genes, tube associated proteins

Genes 29, 48 and 54 of bacteriophage T4, coding for specific tube associated proteins, were cloned to the expression vector pT7-5. The molecular mass of the products of these genes was estimated to be 64, 39 and 36 kDa, respectively. The examined genes are cotranscribed with genes 51, 27 and 28 from the same DNA strand and a common late promoter sequence located downstream of gene 51.

The capsid of bacteriophage T4 is composed of the head which is filled with DNA, a contractile tail, baseplate and six fibers. [1]. The long tail fibers recognize the diglycosyl moiety of lipopolysacharide and enable the virus to bind to the cell wall. It has been well documented that a reversible T4 phage attachment to *E. coli* preceeds the irreversible attachment stage. This first reversible step triggers conformational changes in the baseplate, during which short tail fibers are released and bind irreversibly to the cell wall. Between these two stages of the recognition of the bacterial receptors, the baseplate alone undergoes conformational changes from a thick compact hexagon to a thin, more extended star structure. This rearrangement of the baseplate releases the tail tube which penetrates the cell wall and attaches the incomplete capsid (devoid of the baseplate) to the cytoplasmatic membrane. Such conformational changes can also be induced artificially *in vitro* by treatment of the phage preparation with a 3 M solution of urea or guanidine HCl [2]. The electron microscopic examination of the phage particles induced in such a way revealed an additional unknown structure at the end of the tail tube. This structure was designated as the bulge or sleeve [2]. A detailed biochemical analysis of such preparations has shown that the bulge is composed of the products of genes 29, 48 and probably 54 (the tail tube itself is a multimeric product of gene 19). The accepted pathways of the phage T4 morphogenesis do not include the formation of such a structure [3–5]. Because of the strong interaction of these proteins with phage tail tube (resistance to denaturing agents and elevated temperature) they were designated tube associated proteins [2]. Characterization of particular tube associated proteins can throw some light on the way in which DNA is released from the phage head and transported across the cytoplasmatic membrane, therefore the reconstruction *in vitro* of the bulge structure seems to be of primary importance. As the tail tube preparations themselves cannot serve as a good source of these proteins (small amount and strong complexity) I have turned my attention to the particular genes and their products. These genes were cloned to an overexpressing system and were found to be a satisfactory source of these proteins.

MATERIALS AND METHODS

E. coli [sup D], a permissive host for bacteriophage T4 amber mutants, and *E. coli* Bb a non-permissive host for bacteriophage T4 amber mutants were used for phage growth and plating.

Phages. The amber mutants of bacteriophage T4 used in this study were kind gifts from J. Hageman (U.S.A.) and R. Nivinskas (Lithuania) and are listed in Table 1.

The mutant T4 GT7 containing non-modified DNA was prepared according to Kutter & Snyder [6].

The vector pT7-5 was received from S. Tabor (Harvard Medical School, Boston, Ma. U.S.A.) [7].

Cloning procedures. Most of the restriction enzymes were from New Bio Labs Company (U.S.A.) The ligase and *Eco*RV endonuclease were purified in our laboratory. Cloning was carried out according to Maniatis *et al.* [8].

The complementation test, described previously [9, 10] constitutes a slight modification of the procedures given by Kikuchi & King [3].

The procedure of selective labelling of proteins with ^{14}C-labelled amino acids coded by the cloned T4 DNA fragment, was identical to that described by Nieradko & Podgórska [10] and Schlichholtz *et al.* [11].

SDS/PAGE was performed according to Laemmli [12].

RESULTS AND DISCUSSION

Construction of the hybrids containing genes 28–29 and 48–54

The genes coding for tube associated proteins belong to the cluster of genes whose products are involved in assembling the structure known as the central part of the baseplate. This cluster is located on the bacteriophage T4 map between coordinates 114 and 121.5 and comprises the following genes: 25, 26, 51, 27, 28, 29, 48, 54 [13]. The genes 25, 26, 51, 27, 28 were cloned and expressed in our laboratory [10, 11]. The results of our experiments show that proteins 25 and 26 are coded by a transcript originating from one DNA strand but proteins 51, 27, 28 result from translation of a transcript originating from the opposite DNA strand. As two overlapping late promoters were identified between genes 26, and 51, it is generally assumed that transcription of this cluster of genes is initiated from these promoters. Since no other T4 late promoter sequence was found upstream of gene 51 it was tacitly accepted but never proved that the transcription of the remaining genes of this cluster (genes 29, 48, 54) is initiated from this promoter and that proteins 29, 48, 54 are the result of translation of the common transcript comprising genes 51, 27, 28, 29, 48, 54. However, the possibility of initiation of the transcription of genes 29, 48 and 54 from the opposite strand and the promoter located upstream to gene 54 cannot be excluded. To check both possibilities I cloned the fragments containing genes 28, 29, 48 and 54 into the expression vector pT7-5 in two different orientations to the promoter sequence ϕ10. For isolation of the fragments comprising these genes, DNA isolated from mutant T4GT7 was used (DNA isolated from WT phage is resistant to most restriction enzymes). Such DNA contains non-modified cytosine. The isolated DNA was first digested (on a preparative scale) with *Eco*RV endonuclease. A fragment of 23.3 kb containing genes 28, 29, 48, 54 (coordinates on the genetic map between 115.800–139.000) was isolated from the digestion mixture. Subsequent digestion of the isolated fragment with *Bgl*II endonuclease enabled isolation of a smaller fragment of 3.4 kb, which contained only the genes 28 and 29 (localization between 115.800–119.2 kb). To isolate the segment of DNA with genes 48 and 54, the 23.2 kb fragment was digested with *Pst*I endonuclease. This digestion enabled isolation of the fragment of 2.8 kb (localization between 118.228 and 121.039) which contains genes 48 and 54. The isolated fragments (3.4 and 2.8 kb) were then cloned to the expression vector pT7-5 pre-digested with *Pst*I edonuclease. Before ligation the fragment *Eco*RV-*Bgl*II (genes 28 and 29) and the vector digested with *Pst*I nuclease were treated with mung-bean endonuclease for blunt end generation. The size of the isolated fragments, their orientation and location of the genes on the genetic map are given in Fig. 1. For selection of the transformants containing particular T4 genes the *E. coli* strain Bb non-permissive for T4 amber mutants was used. Particular colonies were tested for complementation of T4 amber

mutants in genes 28, 29, 48 and 54. The results are given in Table 1. The DNA isolated from colonies positive in the complementation test were tested further (*Hin*dIII digestion) for the selection of hybrids with opposite orientations of the cloned fragments (not shown). The orientation of the cloned genes, which is compatible with the natural position of the genes on the genetic map, is represented by fragments designated with the capital letter C in Fig. 1. Recombinants with fragments oriented in such a way were designated as follows: the fragment containing the genes 28 and 29 as pJN1, and the fragment containing the genes 48 and 54 as pJN2. The plasmids with opposite orientation were designated pJN1A and pJN2A, respectively.

Expression of the hybrids pJN1-1A (genes 28–29) and hybrids pJN2-2A (genes 48–54) in *E. coli* BL-21-DE3 and identification of the gene products

Expression of the plasmids containing genes 28 and 29. The selected recombinant clones (pJN1-1A and pJN2-2A) were used to transform *E. coli* strain BL21-DE3 harbouring the T7 RNA polymerase gene. This polymerase, in contrast to most bacterial RNA polymerases, is insensitive to rifampicin and can transcribe

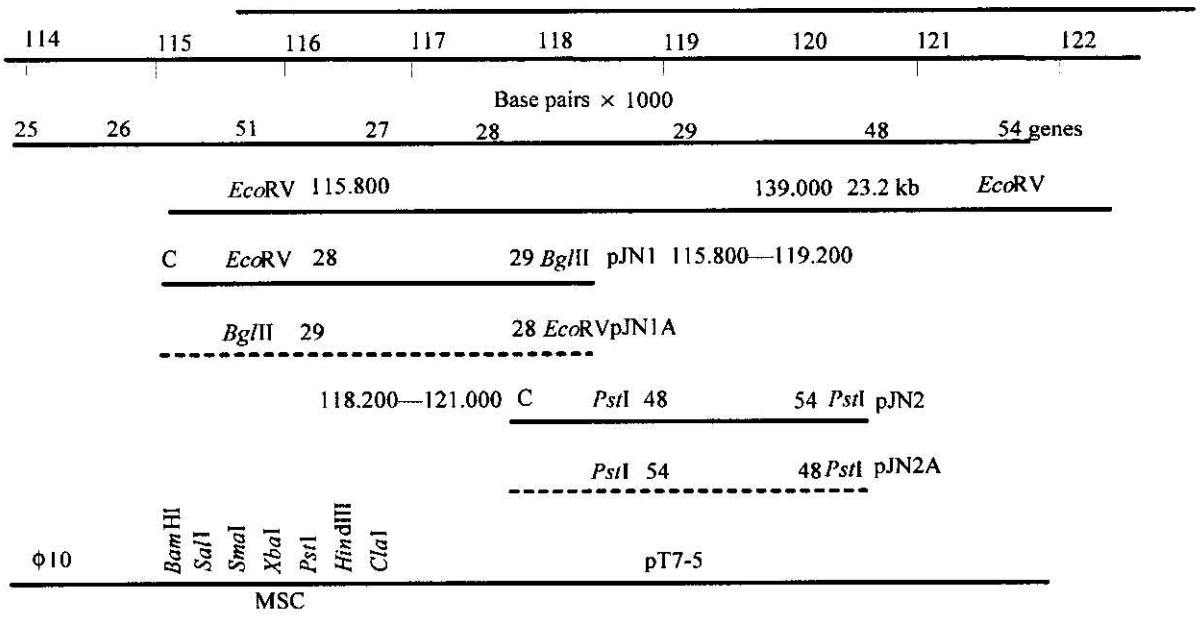

Fig 1. The position of the genes coding for tube associated proteins on the genomic map of bacteriophage T4. The orientation of the cloned fragments compatible with the natural position of particular genes on the genomic map are indicated by a capital letter C (hybrids pJN1 and pJN2). The opposite orientation is marked with a broken line (hybrids pJN1A and pJN2A). The position of multicloning site (MSC) in the pT7-5 vector is shown at the bottom of the Figure. The direction of transcription is indicated with an arrow at the top of the Figure.

Table 1

The results of titration in the complementation test of different T4 amber mutants of E. coli Bb transformed with the hybrids pJN1 and pJN2.

The increased level of titration of particular T4 amber mutants on the non-permissive Bb strain indicated the presence of cloned T4 genes.

Type and source of T4 amber mutants	*E. coli* Bb	*E. coli* CR 64	*E. coli* Bb containing hybrid plasmids	
			pJN1 (genes 28, 29)	pJN2 (genes 48, 54)
28 A412 (R. Nivinskas)	3×10^3	6×10^9	4×10^9	3×10^3
29 Bb, S3 (R. Nivinskas)	4×10^4	3×10^{11}	4×10^{11}	4×10^4
48 NG 284, N85 (J. Hageman)	6×10^3	2×10^{10}	8×10^3	3×10^9
54 NG 154 (J. Hageman)	4×10^3	7×10^{10}	6×10^5	8×10^{10}

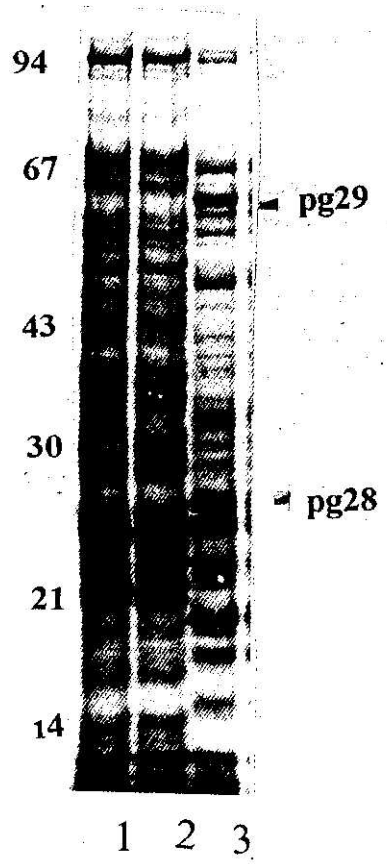

Fig. 2. Optimized protein production system for T4 genes 28 and 29.
E. coli BL21-DE3 harbouring the recombinant plasmids pJN1, pJN1A and pT7-5 vector were grown in minimal medium to $A_{575} = 0.2$, T7 RNA polymerase synthesis was induced by addition of IPTG (isopropylthio-β-D-galactoside) to a final concentration of 10 mM and incubation was continued for 30 min at 37°C. The cells from the induced culture (0.5 ml) were harvested by centrifugation, resuspended in lysis buffer and subjected to electrophoresis. SDS/PAGE gel (10%) was stained with Coomassie Brilliant Blue. Lane 1, BL21-DE3 with plasmid pT7-5; 2, Bl21-DE3 with hybrid pJN1A (reverse orientation of the cloned fragment); 3, BL21–DE3 with hybrid pJN1 (correct orientation of the cloned fragment). The arrows indicate the gene 28 and 29 products. The positions of the protein molecular mass markers (kDa) are shown on the left-hand side of the photograph.

long stretches of the DNA without termination at normal bacterial transcription termination sites [7]. The addition of rifampicin shuts of the bacterial RNA polymerase and enables selective isotopic labelling of phage proteins. For expression of genes 28 and 29 two types of recombinant plasmids pJN1 and pJN1A were used. These plasmids were constructed on the basis of pT7-5 vector into which cloned DNA was placed in two different orientations under the control of T7 RNA polymerase promoter ϕ10.

This allowed for transcription of the phage DNA from both strands. The abundant synthesis of the products of genes 28 and 29 was observed only when strain BL21-DE3 was transformed with hybrid pJN1 (correct orientation) not with the hybrid pJN1A (see Fig. 2, lane 3). Such results clearly indicate that gene 29 is transcribed from the same DNA strand together with gene 28. Previously we have proved that the product of gene 28 is the result of translation of the common transcript which comprise genes 51, 27 and 28 of bacteriophage T4. The transcription of this cluster of T4 genes is initiated from a late promoter sequence located downstream of gene 51. Since no other promoter sequence has been identified between genes 28 and 29 [14, 15] we can assume that the product of gene 29 is the result of translation of the predominant transcript, which comprises genes 51, 27, 28 and 29. The level of synthesis of the products of genes 28 and 29 in the cells examined is very high and there are the dominant proteins, easily seen after Coomassie staining (Fig. 2, lanes 1 and 3) The molecular mass of the gene 29 product was estimated to be 64 kDa and that of the gene 28 products, 24 kDa. Moreover, bands of such molecular mass were observed when proteins were labelled with ^{14}C-labelled amino acids, after rifampicin treatment (not shown).The molecular mass of these products is in agreement with sequencing data for this DNA region [13].

Expression of the hybrids containing genes 48 and 54. Genes 48 and 54 are located on the right-hand end of the second cluster of the baseplate genes. These genes, like to the genes 28 and 29 were placed under control of the promoter of the pT7-5 vector, in two different orientations. The resulting hybrids were designated as follows: hybrid in which cloned fragments assumed an orientation compatible with the natural orientation of the gene on the genetic map of bacteriophage T4, was designated as pJN2 (this orientation is designated with the capital letter C in Fig. 1) that with the opposite orientation, as pJN2A. The elevated level of the protein synthesis, which enabled identification of particular gene products on the polyacrylamide gel, could be observed only when the strain BL21-DE3 was transformed with hybrid pJN2, and not with pJN2A. Such results indicate that genes 48 and 54 are tran-

scribed from the same DNA strand together with the remaining genes of this cluster (genes 51, 27, 28, 29, 48, 54). The transcription of both genes seems to be initiated from the promoter located between genes 26 and 51. Since the cloned fragment contains genetic information for both genes (48 and 54) it was necessary to determine which of the two bands present in the gel (see Fig. 3) can be ascribed to the particular gene. This was done by treating hybrid pJN2 with *Bgl*II endonuclease. Such treatment lead to elimination of the central part of gene 48. The deletion derivate, devoid of the central part of gene 48, lost the ability to complement T4 amber mutants in this gene. Accordingly the upper band in Fig. 3 disappeared when strain BL21-DE3 transformed with such a deletion derivate was examined on the polyacrylamide gel (not shown). This experiment clearly shows that the upper band in Fig. 3 with a molecular mass of 39 kDa must be the product of gene 48, and the lower one, with a molecular mass of 36 kDa, is the product of gene 54.

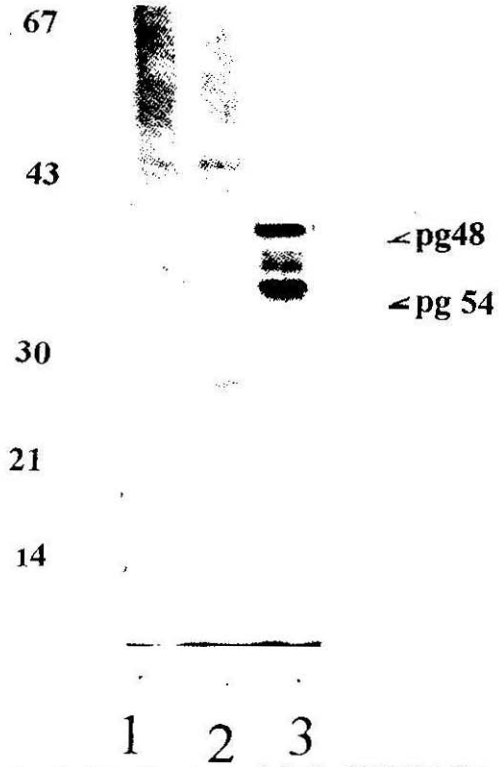

Fig. 3. Autoradiogram of 15% SDS/PAGE of the gene 48 and 54 products synthesized from expressing plasmid pJN2.

E. coli BL21-DE3 were grown in minimal medium to A_{575} = 0.2, then IPTG (cf. Fig. 2) was added to a final concentration of 10 mM; 30 min later the induced culture was treated with rifampicin (250 μg/ml) for 1 h and then labelled for 15 min with ^{14}C amino acids. Lane 1, *E. coli* BL21-DE3 transformed with plasmid pT7-5; lane 2, BL21-DE3 transformed with hybrid pJN2 (the reverse orientation of T4 cloned fragment); lane 3, BL21-DE3 transformed with hybrid pJN2 (correct orientation). The products of genes 48 and 54, unlike the products of genes 28 and 29, were synthesized with lower efficiency and therefore they could only be identified after isotopic labelling which followed the rifampicin treatment.

REFERENCES

1. Goldberg, E. (1983) *Bacteriophage T4*; pp. 32–39, American Society for Microbiology, Washington DC.
2. Duda, L., Gingery, M. & Eiserling, F.A. (1986) *Virology* **151**, 296–314.
3. Kikuchi, K. & King, J. (1975) *J. Mol. Biol.* **99**, 645–672.
4. Kikuchi, K. & King, J. (1975) *J. Mol. Biol.* **99**, 673–694.
5. Kikuchi, K. & King, J. (1975) *J. Mol. Biol.* **99**, 695–716.
6. Kutter, E. & Snyder, L. (1983) *Bacteriophage T4*; pp. 56–58, American Society for Microbiology, Washington DC.
7. Tabor, S. & Richardson, C. (1985). *Proc. Natl. Acad. Sci. U.S.A.* **82**, 1074–1078.
8. Maniatis, T., Fritsch, E.F. & Sambrook, J. (1982) *Molecular cloning. A laboratory manual.* Cold Spring Harbor Laboratory Press, New York.
9. Nieradko, J. & Szewczyk, B. (1990) *Acta Microbiol. Polon.* **39**, 13–22.
10. Nieradko, J. & Podgórska, B. (1993) *Acta Biochim. Polon.* **38**, 313–319.
11. Schlichtholtz, B., Nieradko, J., Gajek, M. & Szewczyk, B. (1991) *Acta Biochim. Polon.* **38**, 311–319.
12. Laemmli, K. (1970) *Nature (London)* **227**, 680–685.
13. Kutter, B., Gutman, B., Mosig, G. & Ruger, W. (1990) *Genetic Map*; pp. 124–261, Cold Spring Laboratory Press Harbor, Cold Spring Harbor, N.Y.
14. Scarlato, V., Storlazi, A. Gargano, S. & Cascino, A. (1989) *Virology* **171**, 475–483.
15. Ishimoto, K., Ishimoto, S., Cascino, A., Cpilaro, S. & Eiserling, F. (1988) *Virology* **164**, 81–90.