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QUARTERLY

Expression of genes 51, 27, 28 coding for proteins of the central part of bacteriophage T4 baseplate in the bacteriophage T7 promoter/RNA polymerase expression system*

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A fragment of T4 DNA (*Xba*I-*Hin*dIII) comprising the genes 51, 27, 28, which encodes the central plug proteins was cloned into plasmid pT7-5 and p7-6 (T7 RNA polymerase expressing system). The examined genes were only overexpressed when the orientation of cloned DNA to promoter Φ10 was as follows: promoter Φ10 and genes 51, 27, 28. This was achieved when the fragment (*Xba*I-*Hin*dIII) was cloned into plasmid pT7-5. Gene 27 and 28 were overexpressed when the intact fragment (*Xba*I-*Hin*dIII) was used. The high rate of the synthesis of proteins 27 and/or 28 had a strong inhibitory effect on the level of synthesis of the product of gene 51. For the overexpression of gene 51 in this system a deletion derivate which was devoid of gene 28 and a larger fragment of gene 27 was prepared.

The genes responsible for the formation of bacteriophage T4 baseplate are clustered in two separate groups [1]. Genes 53, 5, 6, 7, 8, 9, 10, 11 and 12 located between 76 and 91 Kb are conventionally designated as the first group of baseplate genes. The products of these genes with the exception of gene 5 product are assembled into the structure known as the 15S complex. Polymerization of six such complexes "tail to tail " leads to formation of the 70S complex constituing an external part of the baseplate.

The second group of baseplate genes comprises the 25, 26, 51, 27, 28, 29, 48, 54 genes which are clustered between 114 and 121 Kb on the bacteriophage T4 map [1]. The products of these genes with the exception of 25 gene are in turn involved in formation of the central part of the baseplate, commonly designated as the hub or plug [2].

The products of genes of the first cluster constitute 95% of total mass of the baseplate. On the other hand, contribution of the hub proteins to the total mass of baseplate is low. Kikuchi & King [3 - 5] were able to separate and identify proteins of the external part of baseplate, but only one protein encoded by 29 gene from the hub complex. Moreover, the results of the "chasing experiment " of Kozloff [2] did not allow to ascribe genes of the second cluster to individual proteins of the hub complex. Undoubtedly, reconstruction of this structure with the purified proteins would be of importance. For this purpose, however, it is necessary to use an expression system allowing synthesis of these proteins in the amount sufficient for their purification and examination of their properties [6]. The proposal of such a system is the objective of our work. Overexpression of the 25, 26 and 51 genes were reported recently from our laboratory [7]. At present we are describing the expression of *Xba*I-*Hin*dIII fragment comprising besides gene 51 two other genes of the second cluster (genes 27 and 28). The level of

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synthesized products of these genes is sufficient for their purification and further examination of their properties.

MATERIALS AND METHODS

Bacteria. *E. coli* CR63 (*sup D*), a permissive host for T4 amber mutants, and *E. coli* Bb, a non-permissive host for amber mutants, were used for phage growth, streak test and plating.

Phages. The amber mutants of bacteriophage T4 used in these experiments, were kind gifts from W. Wood, J. Hageman, U.S.A. and R. Nivinskas, Lithuania.

Plasmid vectors. pT7-5, pT7-6 were kind gifts from S. Tabor, Harvard Medical School, Boston, Ma, U.S.A. These plasmids contain the Φ10 promoter recognised by T7 RNA polymerase, β-lactamase which is transcribed in the opposite direction to the direction of transcription by T7 RNA polymerase, and a multicloning site. A detailed description of the above plasmids is given by Tabor & Studier [8, 9].

Mutant T4GT7 containing non-modified DNA was propagated according to Kutter & Snyder [10].

Cloning procedures. All restrictive enzymes were from New Biolabs Company, U.S.A. Cloning was carried out according to Maniatis *et al.* [11].

Complementation. Recombinants were tested for the production of phage proteins by complementation tests. In the test, *E. coli* Bb (a non - permissive strain) containing a plasmid with a T4 fragment was infected with an appropriate T4 amber mutant and the increase in bacteriophage titer was determined. The test was termed a complementation *in vivo* when intact cells were used and complementation *in vitro* with the cell extracts. Detailed descriptions are given by Kikuchi & King [3 - 5].

Selective labelling of proteins coded for by cloned T4 DNA fragments. Plasmids pT7-5 and pT7-6 to which genes 51, 27 and 28 were cloned, were used for transformation of *E. coli* BL21-DE3. A few colonies from transformational plates were used to inoculate the minimal medium. When the culture reached $A_{575} = 0.2$ then isopropyl β-thiogalactosidase was added to a concentration of 0.5 mM. After 30 min, rifampicin was added to a final concentration of 500 μg/ml and incubation was continued for 1 h or 40 min. Approximately 10 μCi ^{14}C-amino acids were added and samples were labelled for 15 min. Samples were then subjected to SDS-PAGE electrophoresis [12].

RESULTS AND DISCUSSION

Construction of hybrids containing genes 51, 27 and 28 of bacteriophage T4 baseplate

According to Scarlato *et al.* [13] the second cluster of baseplate genes can be divided into two transcriptional units. The first transcript originates from genes 25 and 26, the second from the remaining six genes of this cluster i.e. genes: 51, 27, 28, 29, 48 and 54. Transcription *in vivo* of this T4 DNA region is initiated from two overlapping late promoters, located between 26 and 51 genes [13]. The overexpression of the genes encompassing the first transcript, genes 25 and 26, and 51 gene from the second transcriptional unit was recently demonstrated in our laboratory [7]. Here we report the cloning and expression of the *Xba*I-*Hin*dIII of T4 DNA comprising genes 51, 27 and 28. This fragment was isolated from the post-digestive mixture of non-modified T4 DNA treated with *Xba*I and *Hin*dIII restriction enzymes. The location of the isolated fragment on the genetic map of bacteriophage T4 is shown in Fig. 1. This fragment was then cloned to plasmids pT7-5 and pT7-6

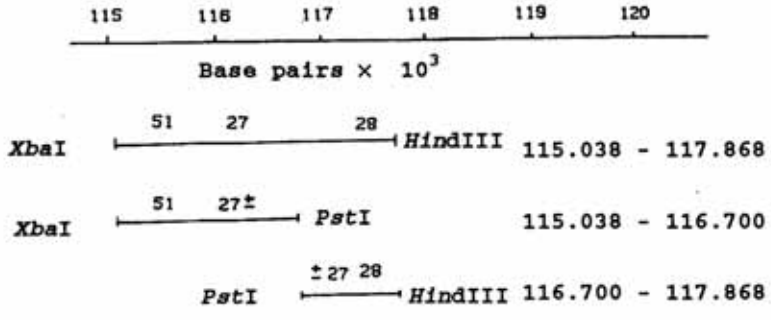

Fig. 1. The position of the cloned XbaI-HindIII fragment and of deleted genes on the bacteriophage T4 genetic map

under control of the bacteriophage T7 Φ10 promoter. Applying the above mentioned pair of vectors enabled transcription of the *XbaI-Hin*dIII fragment from both DNA strands. To enable identification of particular products of phage genes from the initially constructed hybrids the fragments of DNA corresponding to genes 28 and 51 were deleted as illustrated in Fig. 2. The presence of particular genes in cloned fragments was proved by the complementation test Table 1. For such an experiment the resulting hybrids were used to transform a non-permissive *E. coli*, Bb strain, for bacteriophage T4 amber mutants. An increase

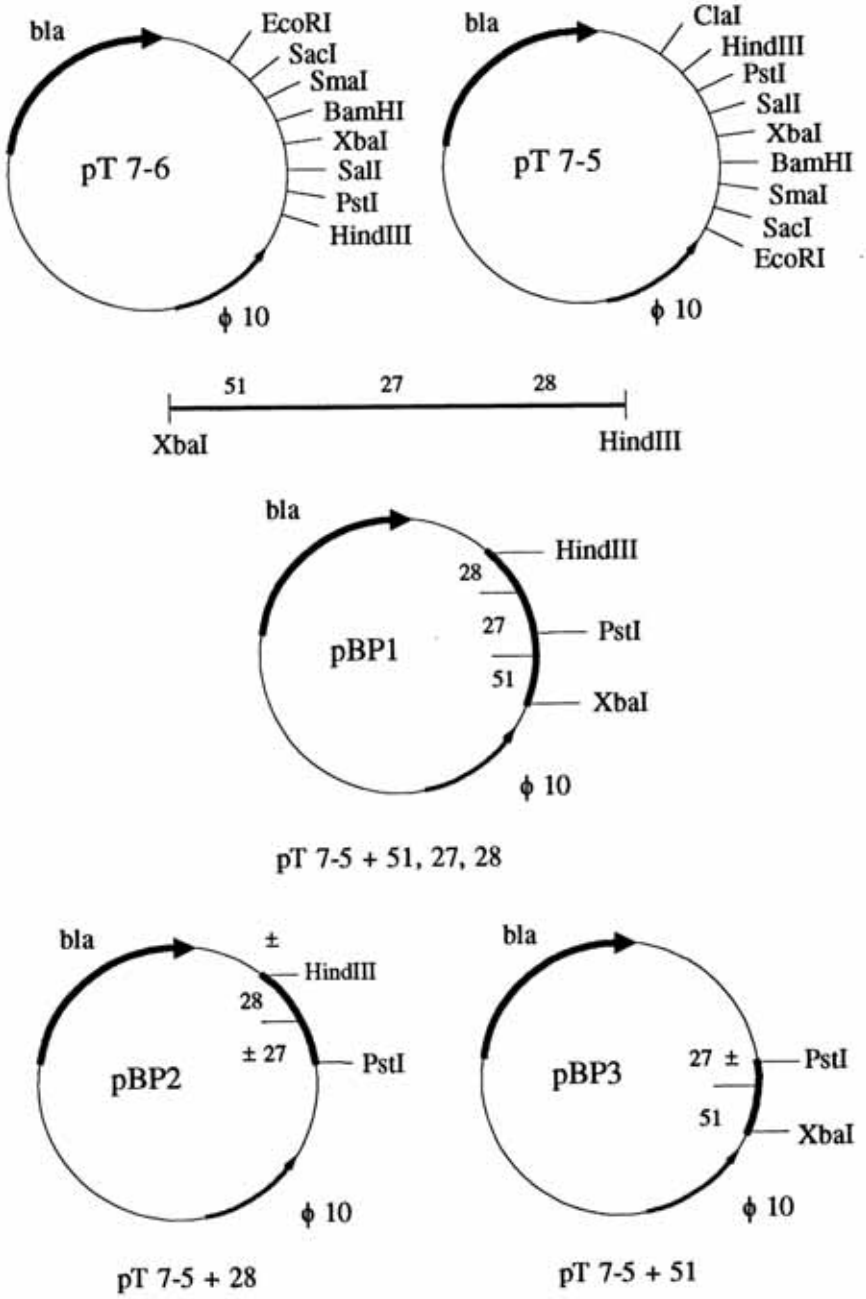

Fig. 2. The diagram of cloning of the T4 XbaI-HindIII fragment to plasmid pT7-5.
The hybrid containing genes: 51, 27, 28 was designated pBP1. This hybrid was then digested with restriction enzymes *XbaI-PstI* (removing the gene 51) and the resulting hybrid was designated pBP2. When restriction enzymes *Pst*I and *Hin*dIII were used (removing the gene 28) the resulting hybrid was designated pBP3. The hybrids constructed on the basis of plasmid pT7-6 (reverse orientation of the multicloning site) were designated as follows: pBP1A containing genes 28, 27, 51; pBP2A containing gene 28, and pBP3A, containing gene 51. The diagram of plasmid pT7-6 is on the left of the Figure (prior to ligation the cohesive ends were eliminated by the action of mung bean nuclease)

in titer to the level of titration on the permissive host for T4 amber mutants indicated synthesis of phage proteins.

Expression of hybrids containing genes: 51, 27 and 28 in *E. coli* BL21-DE3 and identification of particular products of these genes

The hybrids positive in the complementation test were used to transform *E. coli* BL21-DE3 harbouring T7 RNA polymerase gene. After IPTG induction and selective labelling of phage proteins with ^{14}C-amino acids (synthesis of bacterial mRNA was inhibited by addition of rifampicin) the level of synthesis of particular phage products was estimated on polyacrylamide gels presented in Fig. 3, panels A, B, C. The hybrids obtained on the basis of vectors pT7-5 or pT7-6 complemented appropriate T4 amber mutants equally (see Table 1). However, abundant synthesis of phage proteins was observed only under conditions in which orientation of the cloned DNA to the Φ10 promoter was as follows: promoter Φ10 and phage genes: 51, 27 and 28 (see Fig. 3, panels A and B). Such orientation was achieved when the *Xba*I-*Hin*dIII fragment was cloned to the vector pT7-5. The examination of the expression of hybrids containing the intact *Xba*I-*Hin*dIII fragment revealed additionally that the particular phage proteins are synthesized with different efficiency. The most abundant synthesis was observed for the product of gene 28, which can even be seen after Coomassie staining (not shown). On the contrary, the gene 51 product is synthesized in a quantity satisfactory for complementation of T4 amber mutants, but not high enough for visualisation of this product on polyacrylamide gel. This may be due to the inhibitory effect of the products of genes 28 and/or 27 on translation of this region of m-RNA which corresponds to gene 51. To examine this possibility we have attempted to eliminate gene 28 and a part of gene 27 (Fig. 3, panel A, lane 3). We conclude that for the overexpression of gene 51 products deletion of gene 28 and/or 27 is necessary. According to Scarlato *et al.* and Santoro *et al.* [13, 14] gene 51 is accomodated in a long transcript comprising genes 51, 27, 28, 29, 48 and 54. Production of such a long transcript enables regulation of synthesis of particular hub proteins in accordance with actual needs. The products of genes 28 and 27 are structural components of the baseplate [2, 4, 5] and this can explain the necessity of synthesis of higher amounts of both proteins. On the other hand, the product of gene 51 has only a catalytical function [2]. It seems quite probable that for such function a few molecules of this product are sufficient.

Table 1

The results of titration in the complementation test of different T4 amber mutants on E. coli Bb transformed hybrids presented in Fig. 2 (plasmid pT7-5 plus different hub genes). The same results were obtained when plasmid pT7-6 was used for the preparation of hybrids (reverse orientation of multicloning site)

Amber mutants	*E. coli* Bb	*E. coli* CR63	*E. coli* Bb transformed with hybrid		
			pBP1 genes 51, 27, 28	pBP2 gene 28	pBP3 gene 51
51	6×10^5	7×10^{10}	6×10^{10}	3×10^5	5×10^{10}
27	4×10^4	3×10^{11}	1×10^{11}	2×10^4	4×10^4
28	6×10^3	9×10^9	1×10^{10}	8×10^9	4×10^3

We estimated the molecular mass of the product of gene 28 to be 24 KDa, the same value as that inferred from the genetic data and complementation assay [2, 4]. The molecular mass of the product of gene 51 (31 KDa) is in perfect agreement with the data reported earlier [2, 7]. We estimated the molecular mass of the product of gene 27 to be 50 KDa due to the fact that only cell extracts in which a band with such a molecular mass could be demonstrated, have the possibility of complementation of 27 T4 amber mutants. The results reported here conform with the data inferred from the complementation assay reported by Kikuchi & King [4, 5]. The additional band X, which

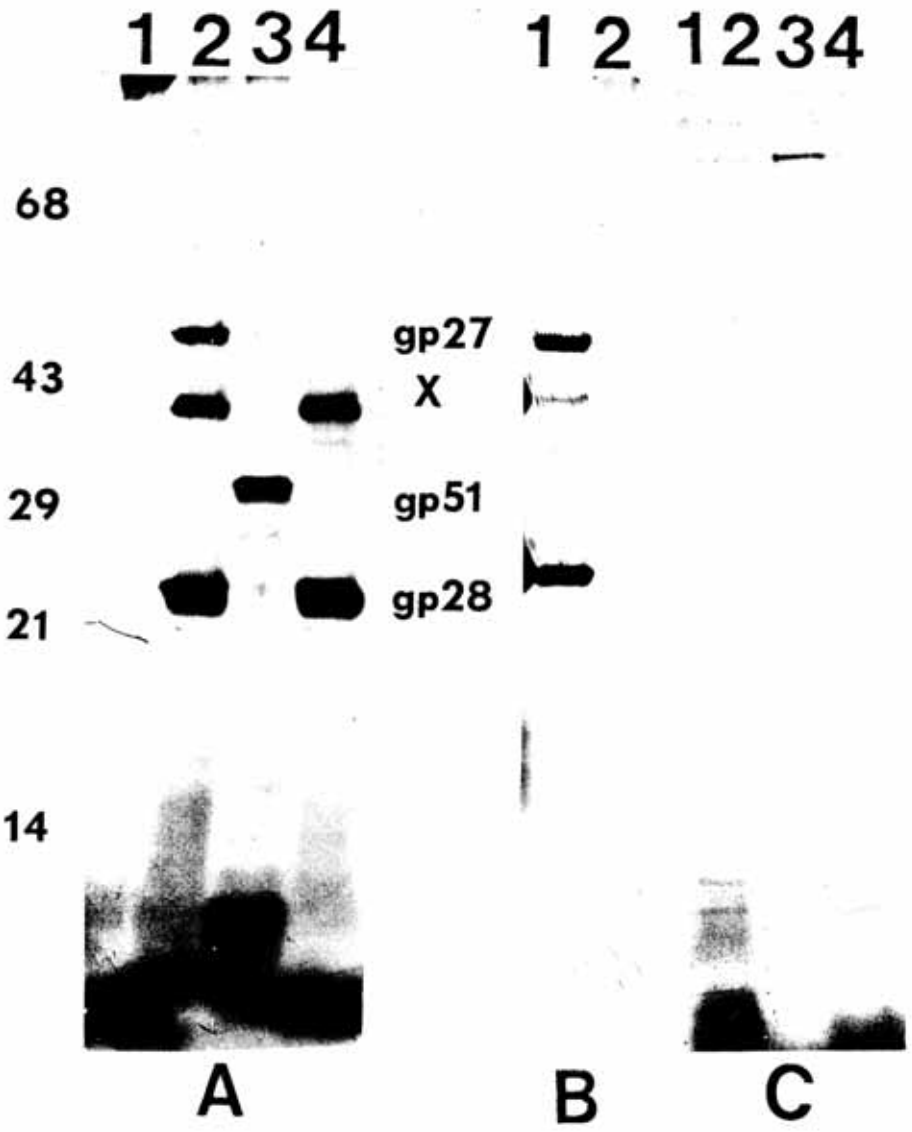

Fig. 3. Synthesis of ^{14}C- amino acids labelled products of genes 51, 27 and 28 in a T7 RNA polymerase system.

The autoradiogram of an SDS-PAGE gel (15% acrylamide concentration). Panel A: 1. BL21-DE3 with plasmid pT7-5 only. 2. BL21-DE3 with hybrid pBP1 (plasmid pT7-5 and genes 51, 27, 28). 3. BL21-DE3 with hybrid pBP3 (plasmid pT7-5 and gene 51). 4. BL21-DE3 with hybrid pBP2 (plasmid pT7-5 and gene 28). All samples were induced for 30 min and treated with rifampicin for 1 h before labelling. Panel B: 1. BL21-DE3 with hybrid pBP1 (plasmid pT7-5 and genes 51, 27, 28). 2. BL21-DE3 with plasmid pT7-5 only. The samples were induced with IPTG for 10 min and rifampicin treatment was restricted to 40 min. Panel C: 1. BL21-DE3 with plasmid pT7-6 only. 2. BL21-DE3 with hybrid pBP1A (plasmid pT7-6 and genes 28, 27, 51). 3. BL21-DE3 with hybrid pBP2A (plasmid pT7-6 and gene 28). 4. BL21-DE3 with hybrid pBP3A (plasmid pT7-6 and gene 51). IPTG and rifampicin as in Panel A. Positions of the protein molecular mass markers (KDa) are shown on the left

usually accompanies the product of gene 27 with molecular mass 38 KDa (Fig. 3, panel A, lane 2) seems to be the result of degradation of this protein. This band was clearly reduced when shorter induction time and rifampicin treatment were applied (10 and 40 min, respectively). For comparison see Fig. 3, panel B lane 1. The band of 38 KDa present in lane 4 panel A could represent an unknown protein coded for by the T4 DNA gene 27 region. However, we think more likely that this band could be the result of translation of an incomplete message

originating from the gene 27 due to elimination of a part of it by *XbaI*- *PstI* digestion.

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