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Molecular Cloning, Nucleotide Sequence, and Promoter Structure of the *Acinetobacter calcoaceticus trpFB* Operon

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The *trpFB* operon from *Acinetobacter calcoaceticus* encoding the phosphoribosyl anthranilate isomerase and the β-subunit of tryptophan synthase has been cloned by complementation of a *trpB* mutation in *A. calcoaceticus*, identified by deletion analysis, and sequenced. It encodes potential polypeptides of 214 amino acids with a calculated molecular weight of 23,008 (TrpF) and 403 amino acids with a molecular weight of 44,296 (TrpB). The encoded TrpB sequence shows striking homologies to those from other bacteria, ranging from 47% amino acids identity with the *Brevibacterium lactofermentum* protein and 64% identity with the *Caulobacter crescentus* protein. The encoded TrpF sequence, on the other hand, is much less homologous to the ones from other species, ranging between 27% identity with the *Bacillus subtilis* enzyme and 36% identity with the *C. crescentus* enzyme. The homologies of both polypeptides are evenly distributed over the entire sequences. The codon usage shows the strong preference for A and T in the third positions typical for *A. calcoaceticus* genes. The *trpFB* operon appears to be unlinked to *trpA*. The *trpFB* promoter has been determined by primer extension analysis of RNA synthesized from the chromosomally and plasmid-encoded *trpFB* operons. The starting nucleotides are identical in both cases and define the first promoter from *A. calcoaceticus*. Potential regulatory features are implied by a palindromic element overlapping the −35 consensus box of the promoter.

The synthesis of tryptophan from chorismate involves five reactions that seem to be conserved among procaryotes. In *Escherichia coli*, five genes are organized in a single operon (27), and *Bacillus subtilis* has six genes in a single operon (8). Unlinked clusters of various *trp* genes, on the other hand, have been found in *Pseudomonas aeruginosa*, *P. putida* (9, 24), *Rhizobium meliloti* (13), and *Acinetobacter calcoaceticus* (22, 23). Thus, the organization of genes encoding the enzymes of tryptophan biosynthesis varies to a great extent among procaryotes (2). Further hints regarding the development of *trp* operons in different species as well as minimal requirements needed for enzymatic activities of the encoded proteins may be obtained from a comparison of nucleotide sequences. Complete nucleotide sequences of *trp* genes are known for *E. coli* (28), *B. subtilis* (8), and *Brevibacterium lactofermentum* (17).

A. calcoaceticus contains seven *trp* genes, which appear to be organized in three unlinked clusters (22, 23). Nucleotide sequences have been reported for the *trpGDC* cluster (15) and for the unlinked *trpE* gene (6). A TrpF protein sequence has been described and compared with other TrpF sequences (18). We report here the cloning and nucleotide sequence of the *trpFB* operon from *A. calcoaceticus*. The results presented here suggest that the *trpA* gene of this species is not linked to the *trpFB* operon. This agrees with the previous observation that linkage of *trpF* to *trpB* appeared to be closer than that to *trpA* (2).

The *trpFB* operon was isolated by direct cloning of a gene library from *A. calcoaceticus* BD4 (14) in *A. calcoaceticus* BD413 *trpB18* (22) by using a newly developed vector for this organism (10). Three clones in which the *trpB18* mutation was complemented were obtained, and the respective plasmids carried insertions of about 22, 16, and 9.3 kilobases, respectively. None of these plasmids yielded complementation of the *trpA* mutation in *A. calcoaceticus* BD413 *trpA23* (22), whereas two of them complemented the *trpF* mutation in *E. coli trpC9830* (F^-) (26). A partial restriction map was made of the plasmid with the smaller insert, and the plasmid was subjected to deletion analysis (Fig. 1). In pWH1754, roughly 3.3 kilobases of the DNA from *A. calcoaceticus* BD4 complemented both *trp* mutations, whereas further deletions in that DNA resulted in loss of complementation. Therefore, this DNA was partially sequenced, and the resulting reading frames were searched for homology to known *trpFB* genes. This led to the identification of the *trp* operon on that DNA (Fig. 1). Then about 2,200 base pairs (bp) were completely sequenced on both strands by the chain termination method (21). The sequence and the open reading frames are shown in Fig. 2. A portion of this sequence was published recently (19). Two open reading frames were found. The first was preceded by a rather poor ribosome-binding sequence around position 262, started at position 274 with ATG, extended over 642 nucleotides, and ended at position 913 with TAA. The encoded polypeptide consisted of 214 amino acid residues, had a calculated molecular weight of 23,008, and showed homology with other *trpF* sequences.

The start of the second gene was somewhat more ambiguous. A continuing coding sequence started at position 838, overlapping with the *trpF* sequence. The first potential start codon would be a GTG at position 899. This was preceded by a rather good ribosome-binding sequence, AAGGAG, at the proper distance. However, another GTG codon at position 917 was only 2 bp downstream from the stop of *trpF*, which is reminiscent of the arrangement of genes in other *trp*

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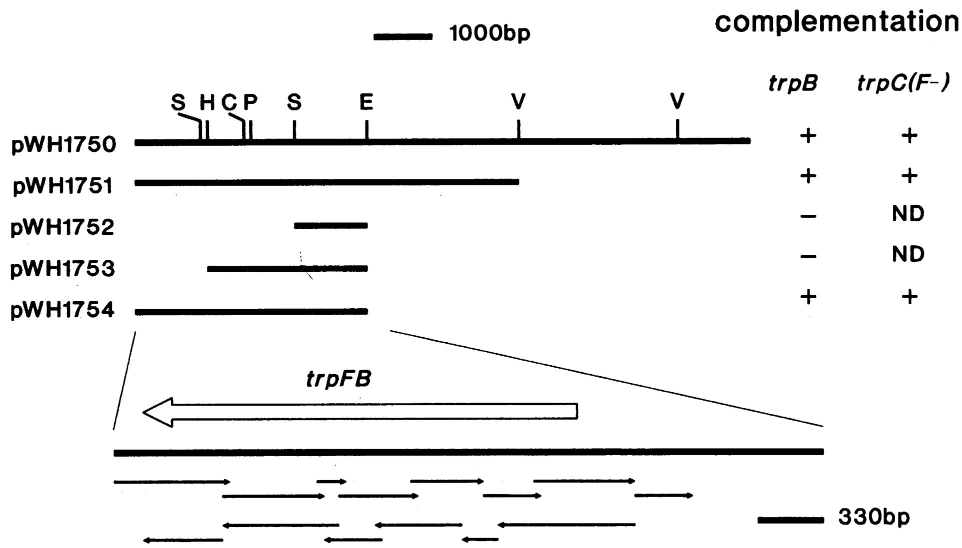

FIG. 1. Partial restriction map, identification by deletion analysis and sequencing strategy of the *trpFB* operon. pWH1750 is the primarily isolated plasmid complementing the *trpB* mutation in *A. calcoaceticus* BD413 *trpB18* (22; indicated in the *trpB* column) and the *trpF* mutation in *E. coli trpC9830* (F^-) [26; indicated in the *trpC*(F^-) column]. pWH1751 contains an *Eco*RV deletion of pWH1750, and pWH1754 contains an *Eco*RI deletion of pWH1751. pWH1752 and pWH1753 contain *Sph*I and *Sal*I-*Hin*dIII deletions of pWH1754, respectively. The constructions make use of the respective restriction sites in the vector portion not indicated in the figure. The solid lines indicate the portion of the insertion that is still present in the plasmids. The figure contains only restriction sites relevant for the indicated constructions. Additional sites are omitted for the sake of clarity. Abbreviations: V, *Eco*RV; E, *Eco*RI; S, *Sph*I; H, *Hin*dIII; C, *Sac*I; P, *Pst*I; ND, not determined. The lower part of the figure shows the sequencing strategy used to determine both strands of the indicated DNA. The open arrow on the top denotes the location and direction of the *trpFB* operon as finally determined by nucleotide sequencing. All methods, including transformation of *A. calcoaceticus* and preparation of plasmid DNA from this species, were done exactly as described previously (10).

operons (8, 28), in particular the *trpGDC* sequence from *A. calcoaceticus* (15). Thus, this codon may well be used to initiate translation. The first ATG codon in this reading frame was located at position 995 and preceded by a poor potential ribosome-binding sequence. However, the other *trp* genes from *A. calcoaceticus* sequenced so far start with an ATG (6, 15). Thus, this ATG may also be used as a start codon. Comparison of the amino acid sequence with those from other organisms (see below) revealed no homology of the residues encoded between the two GTG codons, whereas codons preceding the first ATG led to amino acids with significant homology to other TrpB polypeptides. Based upon this finding, the second GTG seems most likely to be the start codon. The translational start of the *trpB* gene cannot be unambiguously determined at present. For the following analyses it is assumed to be the GTG at position 917. On this basis, the reading frame extends over 1,209 nucleotides to the stop codon TAA at position 2126, encoding a polypeptide of 403 amino acid residues with a calculated molecular weight of 44,296 and striking homologies with other *trpB* genes. The codon usages (data not shown) showed the expected preference for A or T in the third positions, as described for other *trp* genes from this organism (6, 15). The only exceptions were the Val and Thr codons in *trpF* and *trpB* and the Gln codons in *trpB*.

Primer extension analysis (25) was performed with the oligonucleotide with the sequence 5′ ACATCTTGGGAA CGGGTAATACCGC and total RNA prepared from *A. calcoaceticus* BD413 *trpB18* and the same strain transformed with pWH1754 (Fig. 3A). Two main signals flanked by two weaker signals were found for both RNAs, indicating that the transcription start sites of the plasmid- and chromosome-encoded copies are identical. Figure 3B shows the promoter sequence of the *A. calcoaceticus trpFB* operon. The starting nucleotide is probably the A corresponding to the longest primer extension product. The −10 and −35 regions show some homology to the *E. coli* consensus sequence (7). It should be noted however, that the spacing between the boxes would be 19 bp if this assignment were correct. *E. coli* promoters do not contain this spacing (7). The −35 region overlaps with a palindromic element (Fig. 3B). Both the location with respect to the promoter and the palindromic structure support the possibility that this may be a binding site for a repressor protein. It has been shown that transcription of *trpFB* is regulated differently from *trpE* and *trpGDC* (1). In agreement with this result, we did not find homology in the upstream 270 bp with the respective *trpE* or *trpGDC* sequences (6, 15). A potential transcriptional terminator was found between positions 2152 and 2179. This is particularly interesting because it had been suggested that *trpFB* and *trpA* could be cotranscribed (22). If this terminator is functional, it would imply that the *trpA* gene is not contained in this operon. This is further confirmed by additional sequencing data (not shown) extending about 250 nucleotides downstream from the stop of *trpB*, where no reading frame with homology to known *trpA* sequences was found. We thus conclude that *trpA* is not cotranscribed with *trpFB* in *A. calcoaceticus*.

Comparison of the *trpF* and *trpB* coding sequences with the ones from other organisms reveals extensive homologies. Based upon homologies and identities in the amino acid structures the *A. calcoaceticus trpB* gene is most closely related to those from *P. aeruginosa* (5) and *Caulobacter crescentus* (20), followed by those from *B. subtilis* (8), *E. coli* and *Salmonella typhimurium* (4), and *B. lactofermentum* (17). Recently the nucleotide sequences of *trpB* genes from *Bacillus stearothermophilus* (12) and *Thermus thermophilus* (16) and the three-dimensional structure of tryptophan synthetase (11) have been reported. The *trpB* sequence determined here shares all the necessary features for an active

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GCATGCAGTGGCGTAAGTCTAAATGACTTTTTAATATGGTTTACATTTTTACCGTTGCGGGGGCAGCACTGGATTTGCACCAGTTTCCCTAAAGCGAATGCTTTTAACT  110
TGTTACGAATTGTGTAAAGTATAAAGTCTGAGCGAAGATTAAACAATCTGAATACGATCAAATTCGTTCAACTTTGACGCAAAGCACAAAAATTGCATTACAATACTTAG  220
CCCAATGATGGATAGATCGGCTGTCTGTCAGGCAATACAATGAGCTTCTTTCTATGCGAACGCGCGCAAAAATTTGCGGTATTACCCGTTCCCAAGATGTCCAAGCAGCA  330
                                                                         MetArgThrArgAlaLysIleCysGlyIleThrArgSerGlnAspValGlnAlaAla

GTAAGTGCAGGTGCAGATGCCATTGGACTGGTTTTTTCCCACCAAGTCCTCGACATGTTTCTATAGCGCAAGCGCAAGCATTGCTCCAGCATATTCCCGCTTATGTTCA  440
ValSerAlaGlyAlaAspAlaIleGlyLeuValPhePheProProSerProArgHisValSerIleAlaGlnAlaGlnAlaLeuLeuGlnHisIleProAlaTyrValGl

GGTGGTTGGTTTATTTGTGAATGCAACTGCGGATCAAATCAAATCAGTGCTTGATTGTGTGGCTTTGGATGTATTACAACTACATGGCGATGAAACGCCTGAGCAATGTC  550
nValValGlyLeuPheValAsnAlaThrAlaAspGlnIleLysSerValLeuAspCysValAlaLeuAspValLeuGlnLeuHisGlyAspGluThrProGluGlnCysG

AAGAGATTGCTCTGCAGTGCAAGCGTCGCTGGTATAAAGCCATTCAAGTTAAACCAGAGCTTGATGTAGTTGATGAAGTTCAGCGTTATCAGGCCGCTGGTGCAAGTGCG  660
lnGluIleAlaLeuGlnCysLysArgArgTrpTyrLysAlaIleGlnValLysProGluLeuAspValValAspGluValGlnArgTyrGlnAlaAlaGlyAlaSerAla

GTATTGCTGGATGCGTGGCATCCAGAGCTCAAAGGTGGAACTGGTCATCAATTTGATTGGTCGAAGTTTCCCAAGCTGGATATTCCACTTATTCTTGCAGGCGGTTTAAC  770
ValLeuLeuAspAlaTrpHisProGluLeuLysGlyGlyThrGlyHisGlnPheAspTrpSerLysPheProLysLeuAspIleProLeuIleLeuAlaGlyGlyLeuTh

GCCTGAAAATGTTGTAGATGCCATTCAAACCACACACGCTTTTGCAGTGGATGTGAGCGGAGGGGTAGAGGCCGCAAAAGGTATTAAAGATAAACAACTCATCGAACGAT  880
rProGluAsnValValAspAlaIleGlnThrThrHisAlaPheAlaValAspValSerGlyGlyValGluAlaAlaLysGlyIleLysAspLysGlnLeuIleGluArgP

TTATGCAAGGAGTCCAATGTGGATCAGCAAAATAACGTGATTGACTATACGCAATATCCAGATGCTCGTGGGCATTTTGGTATTCATGGCGGACGTTTTGTATCAGAAAC  990
heMetGlnGlyValGlnCysGlySerAlaLysEnd MetIleAspTyrThrGlnTyrProAspAlaArgGlyHisPheGlyIleHisGlyGlyArgPheValSerGluTh

ACTTATGGCGGCACTTGAAGATTTAGAAAATCTTTACAACCGCATGAAAAATGACGAACAGTTTCTGGCAGAATTTGACCGCGATCTTGCCTATTATGTAGGTCGTCCTA 1100
rLeuMetAlaAlaLeuGluAspLeuGluAsnLeuTyrAsnArgMetLysAsnAspGluGlnPheLeuAlaGluPheAspArgAspLeuAlaTyrTyrValGlyArgProS

GTCCACTTTATTATGCTGAACGATGGTCAAAGAAGCTCGGTGGTGCGCAAATTTACTTAAAACGTGAAGACCTGAATCATACAGGTTCACACAAAGTTAATAACACCATT 1210
erProLeuTyrTyrAlaGluArgTrpSerLysLysLeuGlyGlyAlaGlnIleTyrLeuLysArgGluAspLeuAsnHisThrGlySerHisLysValAsnAsnThrIle

GGTCAGGCATTATTGGCCAAGCTTTCTGGCAAAAAACGTATCATTGCAGAAACGGGTGCGGGTCAGCATGGTGTTGCAACTGCAACGATTGCAGCACGTTTGGGCCTCGA 1320
GlyGlnAlaLeuLeuAlaLysLeuSerGlyLysLysArgIleIleAlaGluThrGlyAlaGlyGlnHisGlyValAlaThrAlaThrIleAlaAlaArgLeuGlyLeuGl

ATGTGTCGTGTTCATGGGTGCAGAGGATGTCAAGCGTCAGGCCATGAATGTGTATCGCATGCGTTTGCTTGGTGCGACAGTTATTCCTGTACAAAGTGGTTCAAAAACCC 1430
uCysValValPheMetGlyAlaGluAspValLysArgGlnAlaMetAsnValTyrArgMetArgLeuLeuGlyAlaThrValIleProValGlnSerGlySerLysThrL

TAAAAGATGCCATGAATGAAGCCATGCGCGATTGGGTGACCAATGTAGACAGTACTTATTATGTGATTGGCACTGTAGCAGGTCCACATCCTTATCCTCAGTTGGTGCGT 1540
euLysAspAlaMetAsnGluAlaMetArgAspTrpValThrAsnValAspSerThrTyrTyrValIleGlyThrValAlaGlyProHisProTyrProGlnLeuValArg

GATTTCCAGTCGATTATTGGACGTGAAGCCCGTCGCCAGATTCAGGAACAGGCAGGACGTTTGCCAGATGCGCTTGTGGCGTGTGTCGGTGGTGGTTCTAACGCGATCGG 1650
AspPheGlnSerIleIleGlyArgGluAlaArgArgGlnIleGlnGluGlnAlaGlyArgLeuProAspAlaLeuValAlaCysValGlyGlyGlySerAsnAlaIleGl

GCTGTTTTATCCATTCCTGAATGATCAAGACGTCAAAATGTATGGTGTTGAAGCTGCGGGTCATGGTATCGAAACAGGCAAGCATTCTGCTCCGCTTAATGCAGGGCATG 1760
yLeuPheTyrProPheLeuAsnAspGlnAspValLysMetTyrGlyValGluAlaAlaGlyHisGlyIleGluThrGlyLysHisSerAlaProLeuAsnAlaGlyHisV

TGGGTGTATTACATGGTAACCGCACCTATTTGATGAGTGATCCACAAGGTCAGATTATCGAAACCCACAGTATTTCTGCGGGTCTGGATTACCCTGGTGTTGGCCCTGAG 1870
alGlyValLeuHisGlyAsnArgThrTyrLeuMetSerAspProGlnGlyGlnIleIleGluThrHisSerIleSerAlaGlyLeuAspTyrProGlyValGlyProGlu

CATAGCTTTCTCAAAGACATGCATCGTGTTGAATACGTACCTATTGACGATAACGAAGCATTACAAGGCTTCCGTGACCTTACTCGCATTGAAGGCATTATTCCTGCAAT 1980
HisSerPheLeuLysAspMetHisArgValGluTyrValProIleAspAspAsnGluAlaLeuGlnGlyPheArgAspLeuThrArgIleGluGlyIleIleProAlaIl

CGAGAGTGCTCATGCAATGGCTTATGTCACCAAGCTGGCACCTACCATGGACAAAGATCAGATTATCATTGCCAATGTGTCAGGTCGTGGCGATAAAGACCTAATGACGG 2090
eGluSerAlaHisAlaMetAlaTyrValThrLysLeuAlaProThrMetAspLysAspGlnIleIleIleAlaAsnValSerGlyArgGlyAspLysAspLeuMetThrV

TGGCACGTATTGATGGCATCGAGATGGTAGAAATGTAATTCTAATCATGAACGTGATGTGGAAATGGGCAACATCAATGTGGCCCATTTTTTTGGAGAAAGATAAGCATG 2200
alAlaArgIleAspGlyIleGluMetValGluMetEnd

TTGAGTTTATTTATGGT
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FIG. 2. Nucleotide sequence of the *A. calcoaceticus trpFB* operon. A 2,217-bp sequence of *A. calcoaceticus* DNA is shown, including the *trpF* and *trpB* genes. The encoded amino acid sequences are presented under the nucleotide sequence. Potential ribosome-binding sequences are underlined. The *trpF* reading frame starts at position 274, and *trpB*, which is in the −1 reading frame compared with *trpF*, starts at position 917. Two palindromic sequence elements, one upstream of *trpF* reminiscent of bacterial operators and one downstream of *trpB* reminiscent of bacterial terminators of transcription, are indicated by arrows.

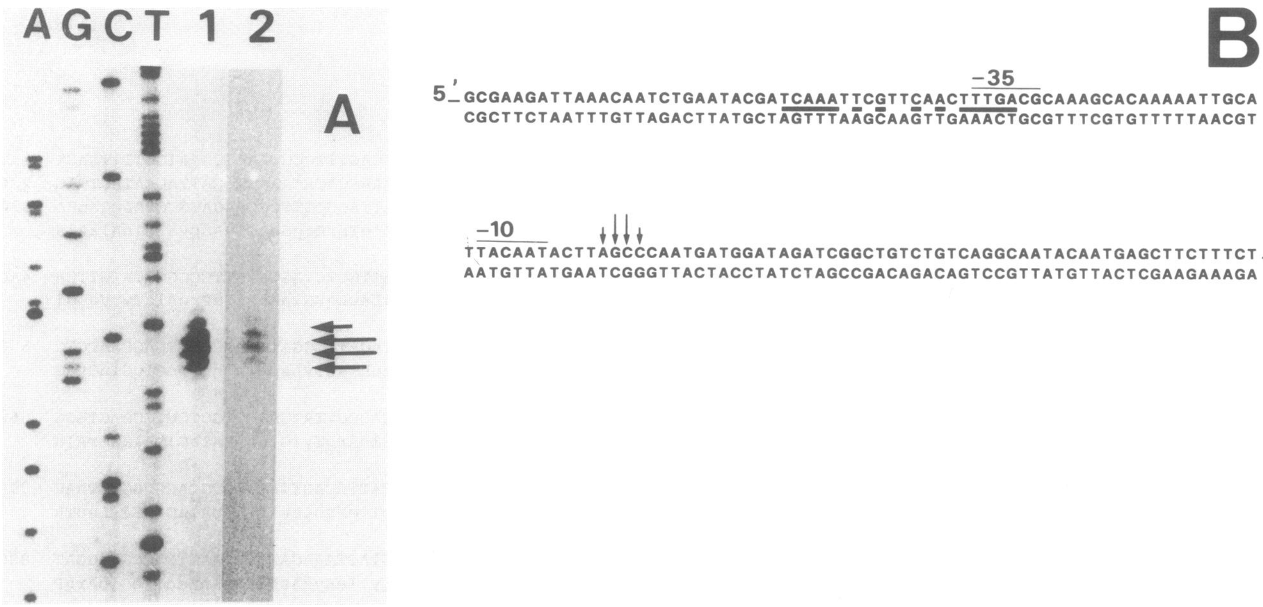

FIG. 3. Primer extension analysis and nucleotide sequence of the *trpFB* promoter. (A) Autoradiograph of the primer extension experiment. The sequencing lanes are labelled A, G, C, and T. Lanes: 1, total RNA from *A. calcoaceticus* BD413 *trpB18* transformed with pWH1754 (4 μg); 2, total RNA from the nontransformed strain (25 μg). The positions of the primer extension products are indicated by arrows; the lengths of the arrows indicate the intensities of the respective bands. Initial experiments in the absence of tryptophan did not reveal a clear regulation of *trpFB* expression on the level of transcription. (B) Signals are aligned with respect to the nucleotide sequence. The proposed −35 and −10 boxes of the promoter are indicated. Furthermore, a palindromic sequence element overlapping partially with the −35 box is indicated by the bold line between the strands.

β-subunit of tryptophan synthetase (16). In particular, the coenzyme-binding amino acids Lys-93, Gly-Gly-Gly-Ser-Asn-Ala (positions 238 through 243), Ser-382, and Gly-383 and the substrate-binding residues Glu-115 and His-121 are located at the predicted positions in the primary structure (11, 16). Although the overall similarities are quite high, with identical amino acids ranging between 47 and 64%, it is surprising that the gram-positive *B. subtilis trpB* gene is more closely related to the gram-negative *A. calcoaceticus* gene than to that from *E. coli*. The same observation has been made for the *trpE* gene (6). The similarities of the *trpF* genes are much less pronounced, with amino acid identity ranging between 27 and 36%. For extensive reviews, consult references 3 and 19. The relatively low homology to the *E. coli trpC(F)* gene differs from the results obtained for *trpB* and *trpE* (6).

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