
A bidirectionally active signal for termination of transcription is located between *tetA* and *orfL* on transposon Tn10

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ABSTRACT

A terminator of transcription with bidirectional activity has been located between the translation termination codons of the genes *tetA* and *orfL* on Tn10. These genes are transcribed towards each other. Each orientation of the intervening sequence is shown to reduce the expression of the *lacZ* and *galK* genes when cloned between the respective structural gene and its promoter. The 3' ends of the respective mRNAs were determined by S1 mapping. The results confirm that the same sequence capable of forming a stem-loop structure with a GC rich stem is the termination signal for both orientations. In the more efficient *tetA* orientation (99%-96% reduction of expression) this sequence is followed by a run of six thymines. In the less efficient *orfL* orientation (96%-78% reduction of expression) it is followed by an AT rich sequence with seven thymines out of eleven base pairs.

INTRODUCTION

The central part of transposon Tn10 contains about 6500 base pairs (1) coding for the genetic markers of this mobile DNA element. Approximately one half of the nucleotide sequence of this core region has been determined (2,3,4) and genetically characterized (5,6,7). The arrangement of the four genes described so far is complex. The genes *tetA* and *tetR* as well as the genes *orfL* and *orfR* start with opposite polarity from a shared regulatory region (4). The genes *tetA* and *orfL* are transcribed towards each other. Their translational termination codons are separated by only 112 base pairs (4,7). Thus, the coding capacity of the characterized Tn10 DNA is completely used by genes with complex, overlapping regions for the regulation of their expression (4,8).

The DNA sequence between *tetA* and *orfL* contains a region with the potential of forming a stem-loop structure with a GC rich stem. In the direction of *tetA* this sequence is followed by an oligo T region and in the direction of *orfL* by a thymine rich sequence with seven thymines out of eleven base pairs. Thus, there is a typical transcription termination signal in the

tetA polarity (9). A recent review of terminator sequences reveals that some deviations from the oligo T consensus sequence may still render a functional terminator (10). Therefore, this termination signal may function bidirectionally, thus avoiding possible mutual interference of transcription of tetA and orfL and the possible interference of complementary transcripts in the translation of either mRNA (11).

Although terminator activity is generally unidirectional (12), some terminators have been considered to function bidirectionally. In particular the fd terminator, which also has an AT rich sequence preceding the stem loop feature was shown to function in both orientations (13,14). Recently, a bidirectional activity was proposed on the basis of nucleotide sequence data for the tonB (15) and rrnD terminators (12). In this communication we demonstrate the termination of transcription within the 112 base pairs between tetA and orfL in both orientations in vivo using suitable indicator plasmids. In addition, we determined the 3'ends of both in vivo mRNAs by nuclease S1 mapping.

MATERIALS AND METHODS

Materials

Restriction endonucleases, T4 DNA ligase, nuclease S1, and DNA-polymerase I large fragment were purchased from BRL, Bethesda, Maryland. α - ^{32}P dATP, $^{32}\text{P}_i$, and ^{14}C galactose were from Amersham, Braunschweig. γ - ^{32}P ATP was synthesized from ADP and $^{32}\text{P}_i$ by a published procedure (16).

Construction of plasmids

The 324 bp HaeIII fragment was prepared from pRT61 (5) by elution from a 5% polyacrylamide gel as described (17). The preparation of plasmid DNA was as described (18). pRT405 (kindly provided by Drs. L.V. Wray, Jr., L. Munson, and W.S. Reznikoff) was digested with SalI and the protruding ends were filled in as described (19). The 324 bp HaeIII fragment was ligated into the filled in SalI site and transformed to E.coli CSH26 as described (18). Recombinants were screened using their white colour on lactose McConkey agar. pWH 951 gave white colonies for several days. pWH 961 gave white colonies which eventually turned pink. The orientation of the insertion was confirmed by double digestions with BamHI and ClaI. The ClaI site is located at position 1206 in the tetA gene which is 101 bp from the respective end of the 324 bp HaeIII fragment (2). pRT405 contains two BamHI sites spanning the 146 bp fragment containing the tet P_A promoter which was

inserted six bp to the left of the SalI site indicated in Figure 2. The 324 bp HaeIII DNA was also cloned into the SmaI site of pDS26t (kindly provided by Dr. W. Schumann) using the same procedures. The host for these constructions was E.coli N100 (20). Transformants were screened by their white colour on galactose McConkey agar (20). pWH 952 gave white colonies which turned red after a couple of hours. pWH 962 resulted in white colonies which turned red even faster. Thus, in our hands the lacZ system was superior for screening terminator activity. The orientations of the insertions were confirmed by digestion with HindIII and ClaI. pDS26t contains a single HindIII site 18 bp upstream of the SmaI site indicated in Figure 2.

Enzyme activity assays

The β -galactosidase assay was exactly done as described by Miller (22). The assay for β -galactokinase activity was exactly done as described by McKenney et al. (20). The units were calculated using the following formula:

$$U = \frac{\text{cpm} \times 100 \times 1.2 \times \text{dilution}}{\text{spec.act.} \times 2.22 \times 10^3 \times 50} \quad \frac{\text{nmole}}{\text{min} \times A_{578}}$$

Because the ^{14}C galactose had a specific activity of 0.5 $\mu\text{Ci}/\mu\text{Mole}$ the units are nmole galactose-1 phosphate formed per minute and per 1 OD cells.

S1 mapping

The preparation of total RNA and the S1 mapping was done as described by Aiba et al. (21). The phenol was preheated to 60°C for both extractions in this procedure. The DNA-RNA hybrid was treated in five steps with increasing amounts of nuclease S1 from 50 U to 250 U for 15 min. The hybridization temperature was varied between 37°C and 28°C. The DNA probes were prepared from the plasmids pWH951 and pWH962. pWH951 was completely digested with BamHI, 3'end labelled by filling in the protruding ends with dGTP and α ^{32}P dATP using the large fragment of DNA polymerase I (17), redigested with Hind III, and the respective DNA fragment was eluted from a 5% polyacrylamide gel (17). pWH962 was digested with HindIII, filled in as above using dTTP and α ^{32}P dATP, redigested with TaqI and the respective DNA fragment was eluted from a 5% polyacrylamide gel as above. These DNAs were used as probes and part of it was taken for a G+A reaction according to Maxam and Gilbert (17) to serve as size markers. The products of the S1 reaction and the size markers were separated on denaturing polyacrylamide

gels and autoradiographed as described before (4). The S1 maps were determined twice using three different S1 concentrations ranging from 50 to 1000 units in each experiment.

RESULTS AND DISCUSSION

Termination activity between *tetA* and *orfL*

In order to measure the efficiency of termination of transcription between *tetA* and *orfL* on Tn10 we cloned a DNA fragment containing this sequence into suitable terminator screening plasmids (20; Wray, Munson, and Reznikoff, personal communication). Figure 1 describes the location of this DNA with respect to the genetic structure of Tn10 (4). It was isolated as a 324 bp *Hae*III fragment from pRT61 (5) spanning from position 1105 to position 1429 of the published sequence (2).

The first termination indicator system used in this study is based on pRT 405 (Wray, Munson, and Reznikoff, personal communication) which contains the *lacZ* gene under transcriptional control of the *tet* P_A promoter from Tn10 (8). Between promoter *tet* P_A and the *lacZ* structural gene is a single *Sal*I site which was used to insert the 324 bp *Hae*III DNA in both orientations. Figure 2 outlines this construction. The resulting plasmid containing the *tetA* polarity of the terminator is called pWH951 and the plasmid containing the *orfL* polarity of the terminator is called pWH961. Both recombinant plasmids contain the inserted DNA between reconstructed *Sal*I sites.

E.coli CSH26 was used as a host strain to determine the plasmid encoded

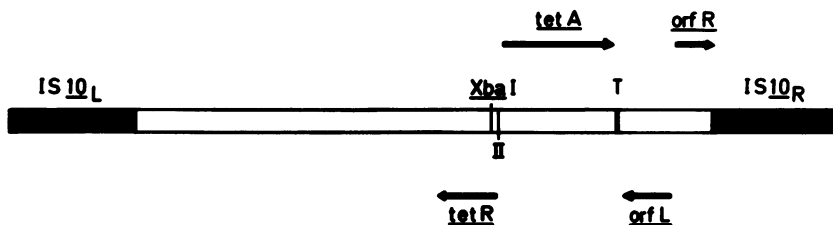


Fig. 1: Location of characterized genes in the core of Tn10. The transposon Tn10 is shown schematically. The flanking inverted repeats are filled. The central core DNA is 6500 bp long and contains the genes indicated by arrows. The *Xba*I and *Minc*II (II) sites are indicated to relate this drawing to the restriction map. The terminator structure between *tetA* and *orfL* is indicated and marked T. References are given in the text.

Table I: β -galactosidase activity expressed by different constructions

plasmids	β -gal activity	stand. dev.	% of maximum
pRT405	1713	64	100.0
pRZ5202	8	1	0.5
pWH951	21	4	1.2
pWH961	118	17	6.7

β -gal activity was measured as described and is given in units as defined by Miller (22). *E.coli* CSH26 was used as an indicator strain.

lacZ activity. It was transformed with pRT405, pRZ5202, a promoterless derivative of pRT405 lacking lacZ gene expression (Munson and Reznikoff, personal communication), pWH951, and pWH961. The activity of β -galactosidase expressed by these strains is given in Table I. *E.coli* CSH26/pRT405 produces about 1700 units of β -gal activity (22) representing the maximal expression. The background activity is given by the strain with pRZ5202 with 0.5% of the maximal expression. Insertion of the terminator in the tetA orientation reduces the maximal expression to 1.2% and insertion of the terminator in orfI polarity reduces it to 6.7% of the maximal value (Table I). This result indicates that the tetA terminator may be active in both orientations. However, it has been shown, that a reduced expression can also be due to interference of the secondary structure of the mRNA with initiation of translation (23). Although inspection of the sequence of this construction (2,24) did not confirm this assumption, we tested the termination activity of the tetA terminator in an independent experiment.

For this purpose the 324 bp HaeIII DNA was cloned into the single SmaI site of pDS26t. This plasmid contains the galK gene under transcriptional control of the gal promoter. Insertion in the Sma I site allows screening of terminator activity (20). The resulting plasmids are also shown in Figure 2. pWH952 contains the terminator in the tetA orientation and pWH962 in the orfI polarity. These plasmids were transformed to *E.coli* N100 and the galactokinase activity of the recombinant strains was determined (20). The results are presented in Table II. pDS26t leads to a galactokinase activity of 427 U representing 100% expression. *E.coli* N100 without a plasmid yielded 0.5% expression of the maximal value. The terminator in the tetA orientation reduces expression of galactokinase to about 4%, and in the orfI polarity the reduction is to about 22% compared to pDS26t (Table II).

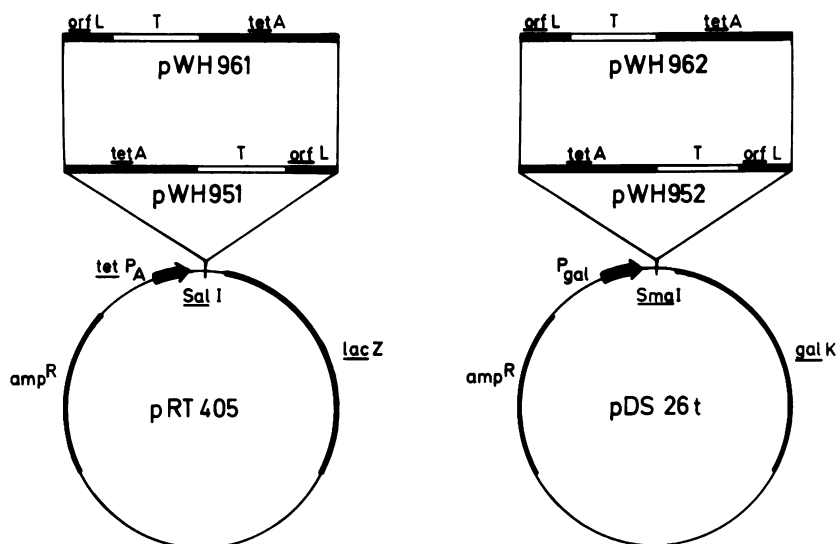


Fig. 2: Plasmids for the analysis of terminator activity. The recombinant constructions based on pRT405 (left side) and pDS26t (right side) are shown. The inserted DNA is full black to indicate the ends of the orfL and tetA structural genes. The open sequence between those genes contains the signal for termination of transcription.

This result may also be interpreted as transcriptional terminator activity of the 324 bp HaeIII fragment in both orientations.

The results obtained in both experiments agree very well. Both show significant reduction of expression of the respective indicator gene. In both determinations the residual expression of the respective indicator gene behind the orfL orientation is about 5.5 fold higher as compared to the tetA orientation. This observation may explain previous results indicating that a strong promoter transcribing orfL is able to read through to the tetR

Table II: Galactokinase activity expressed by different constructions

Plasmid	gal K activity	stand. dev.	% of maximum
pDS26t	427	59.8	100.0
none	2.0	0.6	0.5
pWH952	18	3.3	4.2
pWH962	96	5.7	22.5

galactokinase activity was measured as described by McKenney et al. (20). The units are defined in Materials and Methods. E.coli M100 was used as an indicator strain.

gene (25). The relative efficiency of both orientations is the same in these experiments (Tables I and II). However, the level of residual expression of the galactokinase gene is about 3.5 fold higher than the residual expression of β -galactosidase.

Mapping of the termination nucleotides

In order to relate the obvious termination of transcription indicated by the results in Tables I and II to the terminator structure on the 324 bp HaeIII fragment originating from Tn10 we determined the 3' ends of the mRNAs by nuclease S1 mapping.

The total RNA from E.coli CSH26 transformed with pWH951 was used to map the termination point in the tetA orientation. The DNA probe was prepared from pWH951 by digestion with Bam HI, 3' end labelling, followed by redigestion with HindIII, and elution of the respective DNA from a polyacrylamide gel. Part of this DNA was used as a probe for the mRNA and the other part was cleaved at the purines to serve as a size marker. The result is displayed on the left side of Figure 3. The end point of the tetA mRNA is at position 1332 on the 5' side of the oligo T run and the 3' side of the stem-loop feature of the sequence (2). This result is in contradiction to the termination points found at the 3' side of the oligo T run in other systems (10). Lowering the final hybridization temperature from 37°C to

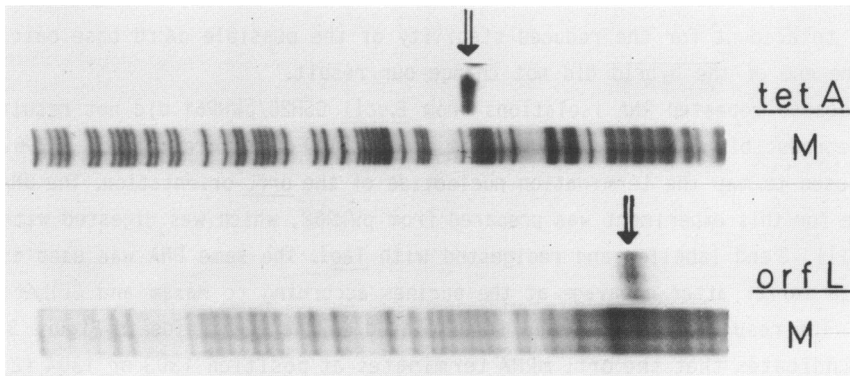


Fig. 3: S1 mapping of the termination nucleotides. The result of the nuclease S1 maps for the 3' ends of two mRNAs are shown. The left side shows the length of the hybrid formed in the tetA orientation. The right side shows the length of the hybrid formed in the orfL orientation. The lanes M contain G+A reaction products of the respective DNA probes as size markers. The origin of the DNA probes and mRNAs is described in the text.

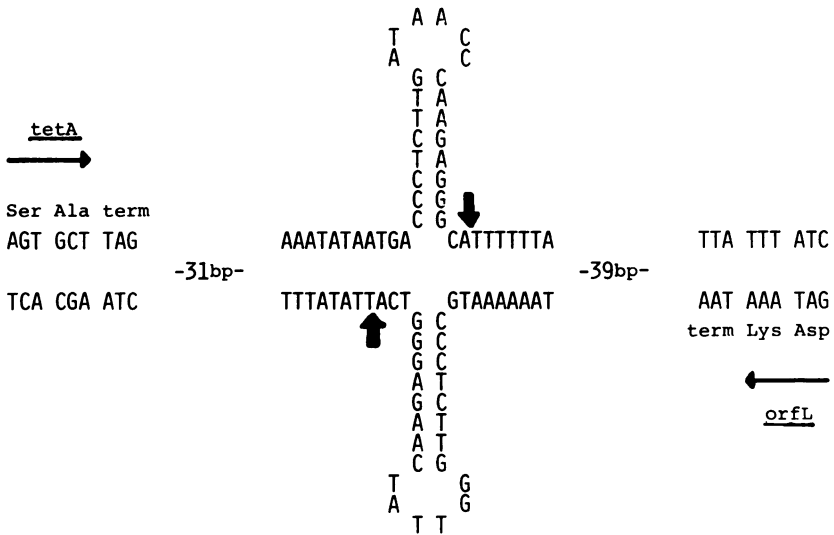


Fig. 4: Nucleotide sequence interpretation of the termination events. The DNA sequence is shown for the terminator DNA between tetA and orfL in the potential structure that might be adopted by the mRNA. The translation stop codons for tetA and orfL are indicated as well as their distance from the stem loop structure. The heavy arrows indicate the termination nucleotides as determined by nuclease S1 mapping.

28°C to account for the reduced stability of the possible dA•rU base pairs at the end of the hybrid did not change our result.

Because repeated RNA isolations from E.coli CSH26/pWH961 did not result in reproducible S1 maps the RNA from E.coli N100 transformed with pWH962 was used to map the termination nucleotide of the orfL orientation. The DNA probe for this experiment was prepared from pWH962, which was digested with HindIII, 3'end labelled and redigested with TaqI. The same DNA was used as a size marker after cleavage at the purines according to Maxam and Gilbert (17). The result of this S1 map is displayed on the right side in Figure 3. It indicates that the orfL mRNA terminates at position 1303 or 1304 (2) irrespective of lowering the final hybridisation temperature from 37°C to 28°C.

The results from both experiments are shown in relation to the assumed tetA terminator sequence in Figure 4. The termination codons of the tetA and orfL reading frames are also indicated in this figure. It may be taken from Figure 4 that both termination points appear to be at identical posi-

tions with respect to the polarity of transcription and the stem loop feature indicated in the figure. The results from the S1 mapping confirm the conclusion that termination of transcription is the reason for reduced expression of both indicator genes used in this study. The sequence interpretation in Figure 4 places the endpoints of the mRNAs closer to the GC rich stem loop feature than has been reported for other terminators (9,10). This may be due to the instability of the dA·rU double strand at the end of the hybrid. At reduced S1 concentrations the 3' ends of the mRNAs mapped about three nucleotides further downstream. It has been reported that S1 mapping results can be ambiguous regarding the exact end nucleotide of the mRNA (27). Thus, no further attempt was made to verify the precise *in vivo* 3' nucleotide of the mRNAs. However, the S1 mapping demonstrates clearly, that the termination event is structurally related to the stem-loop feature of the sequence displayed in Figure 4.

The genetic structure of the core region of Tn10 outlined in Figure 1 suggests that a possible bidirectional termination of transcription between genes tetA and orfL may be of advantage for the expression of these genes. Whereas the regulation of expression and function of the orfL gene product is not yet known, it is clear, that the gene tetA mediating resistance against the antibiotic tetracycline must be expressed very efficiently upon induction by the drug (1,8,26). This would be hampered by transcription of both DNA strands as well as by the presence of a complementary transcript interfering with translation (11). Furthermore, nucleotide sequence analysis reveals no open reading frames on the non-coding strands of the tetA and orfL structural genes (2,4). Transcription of orfR and tetR is initiated by their own promoters located in the 5' regions of the respective genes (4,8). Therefore there is no biological reason that the non coding strands of tetA and orfL should be transcribed.

The transcription terminator displayed in Figure 4 is in both orientations preceded by translational stop codons in all three possible reading frames (2). This is in agreement with bidirectional termination of transcription at this sequence.

We have not attempted to determine the rho factor dependence of termination in this system. Because the tetA orientation has a perfect agreement with the rho factor independent terminators, the rho contribution is probably negligible for this polarity. The fact that the orfL orientation yields a clear S1 map with a termination point behind the stem loop structure indicates that rho independent termination occurs at this position.

Some contribution of rho dependent termination is well possible because the S1 mapping signals turned out to be weaker than the ones for the tetA polarity. This assumption is also deduced from the comparison with the fd terminator where the lack of consensus to the oligo T run in the reverse orientation leads to an increased rho contribution (14). Taken together the results presented in this article demonstrate that a bidirectionally active termination signal separates the genes tetA and orfL on transposon Tn10.

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