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The *tac* promoter: A functional hybrid derived from the *trp* and *lac* promoters

(hybrid promoters/controlled gene expression/promoter efficiency/synthetic DNA/portable Shine–Dalgarno region)

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ABSTRACT **Two hybrid promoters that are functional in *Escherichia coli* have been constructed. These hybrid promoters, *tac*I and *tac*II, were derived from sequences of the *trp* and the *lac* UV5 promoters. In the first hybrid promoter (*tac*I), the DNA upstream of position −20 with respect to the transcriptional start site was derived from the *trp* promoter. The DNA downstream of position −20 was derived from the *lac* UV5 promoter. In the second hybrid promoter (*tac*II), the DNA upstream of position −11 at the *Hpa* I site within the Pribnow box was derived from the *trp* promoter. The DNA downstream of position −11 is a 46-base-pair synthetic DNA fragment that specifies part of the hybrid Pribnow box and the entire *lac* operator. It also specifies a Shine–Dalgarno sequence flanked by two unique restriction sites (portable Shine–Dalgarno sequence). The *tac*I and the *tac*II promoters respectively direct transcription approximately 11 and 7 times more efficiently than the derepressed parental *lac* UV5 promoter and approximately 3 and 2 times more efficiently than the *trp* promoter in the absence of the *trp* repressor. Both hybrid promoters can be repressed by the *lac* repressor and both can be derepressed with isopropyl β-D-thiogalactoside. Consequently, these hybrid promoters are useful for the controlled expression of foreign genes at high levels in *E. coli*. In contrast to the *trp* and the *lac* UV5 promoters, the *tac*I promoter has not only a consensus −35 sequence but also a consensus Pribnow box sequence. This may explain the higher efficiency of this hybrid promoter with respect to either one of the parental promoters.**

The DNA sequences of many prokaryotic promoters (reviewed in refs. 1–3) are known, yet very little is known about which features in these sequences determine the efficiency of promoters. Two domains upstream of the start site of transcription have been identified for which a consensus sequence has been formulated (1–5). These domains are the −35 sequence (5′-T-T-G-A-C-A) and the Pribnow box (5′-T-A-T-A-A-T) in the −10 region. Both domains are in close contact with the RNA polymerase during initiation of RNA synthesis (2, 6). Almost all promoter mutations map in or near these domains (reviewed in ref. 1).

The relative efficiencies of only a few promoters have been measured (7). We have determined the relative efficiencies of four promoters and we show that the efficiency of the *lac* UV5 promoter can be greatly increased by replacing its −35 region with the −35 region of the stronger *trp* promoter. Here, we describe the construction and the properties of the hybrid promoters *tac*I and *tac*II.

MATERIALS AND METHODS

Bacterial Strains. For standard transformations *Escherichia coli* 294 (ref. 8) was used routinely. The *lac*-repressor-overproducing strain used in this study was *E. coli* D1210, which was kindly provided by J. R. Sadler (9). This strain carries the $lacI^q$ and $lacY^+$ alleles on the chromosome but otherwise is identical to *E. coli* HB101 [F^- $lacI^+$, $lacO^+$, $lacZ^+$, $lacY^-$, gal^-, pro^-, leu^-, thi^-, end^-, hsm^-, hsr^-, $recA^-$, $rpsL^-$] from which it was derived (9).

Galactokinase assays were done using *E. coli* C600 ($galE^+$, $galT^+$, $galK^-$, lac^-, thr^-, leu^-). This host was kindly provided by M. Rosenberg (7). The *trp* repressor-minus host we used was *E. coli* W3110$trpR^-$. The Tn*10*-containing host used for the strain constructions described below was *E. coli* K582 ($trpR^+$, *thr*::Tn*10*; from H. Miller).

Source of Plasmids. The sources of the *trp* promoter and the *lac* promoter were the plasmids pHGH207-1 and pHGH107-11, respectively. Both plasmids were constructed in this laboratory as described (10, 11). For measurement of promoter efficiencies the plasmid pKM-1 was used (7, 12). This plasmid was kindly provided by M. Rosenberg.

Plasmid Constructions. The procedures for isolation of plasmid DNA, cleavage with restriction enzymes, isolation of DNA fragments, kinase treatment of DNA fragments, ligation with T4 DNA ligase, and transformation of *E. coli* were standard procedures as used in this laboratory (8, 10, 11).

Assays. Human growth hormone (HGH) levels in cells were determined by using a radioimmunoassay as described (8). The galactokinase levels were measured as described in ref. 12.

Strain Constructions. In order to measure the activity of the *trp* promoter in the *galK* system we had to construct a derivative of C600 $galK^-$ that lacks a functional *trp* repressor. This was done as follows. *E. coli* K582 $trpR^+$, *thr*::Tn*10* harbors Tn*10* in the *thr* gene, which is close to the *trpR* gene, whose map position is at 99.5 min. By using phage P1 transduction the *thr*::Tn*10* region of K582 was transduced into W3110$trpR^-$. Tetracycline-resistant colonies that were still $trpR^-$ (resistant to 5-methyltryptophan at 100 μg/ml) were isolated. Strain HDB1 ($trpR^-$, *thr*::Tn*10*) was obtained in this manner. This Tn*10* region was subsequently transferred into C600 $galK^-$ by using P1 transduction. Tetracycline-resistant colonies that were resistant to 5-methyltryptophan were isolated. HDB2 ($galE^+$, $galT^+$, $galK^-$, $trpR^-$, *thr*::Tn*10*, lac^-, thr^-, leu^-) was obtained in this way. This strain was used to determine the *trp* promoter efficiency in the absence of the *trp* repressor.

RESULTS

Construction of *tac*I. The construction of *tac*I as shown in Fig. 1 has been described in detail by us in a previous communication (11). The DNA sequence of the resulting *tac*I promoter in the expression plasmid pHGH807*tac*I is shown in Fig. 2. In this expression plasmid, the *tac*I-containing fragment en-

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Abbreviations: HGH, human growth hormone; bp, base pair(s).

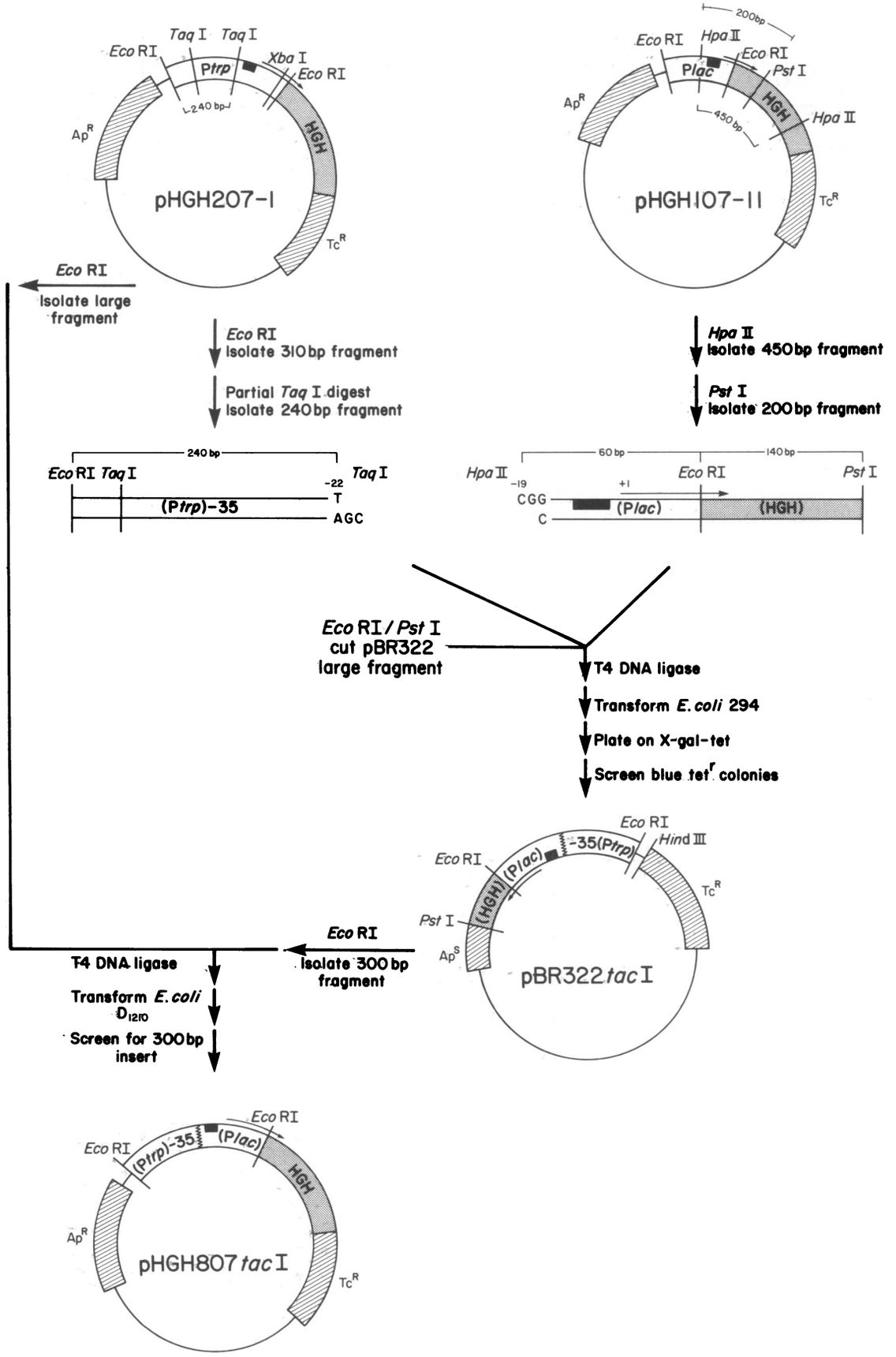

FIG. 1. Construction of pHGH807*tac*I containing the *trp–lac* hybrid promoter *tac*I. P*trp* and P*lac*, promoters for *trp* and *lac*; Ap, ampicillin; Tc or tet, tetracycline; X-gal, 5-bromo-4-chloro-3-indolyl β-D-galactoside; bp, base pair(s).

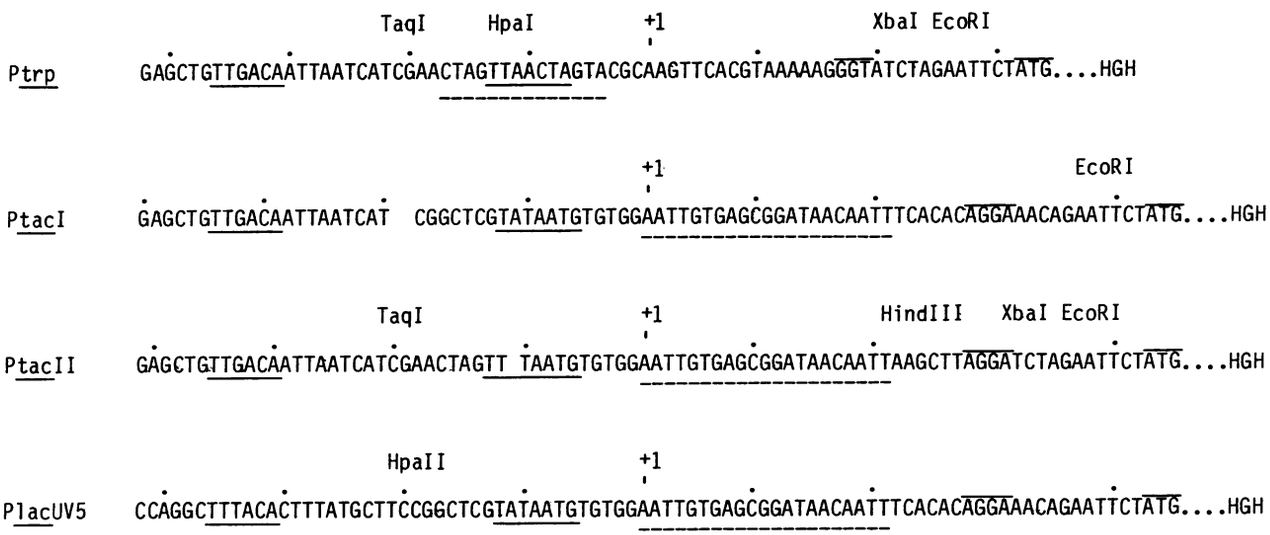

FIG. 2. DNA sequences of the *trp*, *lac* UV5, *tac*I, and *tac*II promoters. The −35 sequence and the Pribnow box sequence of the promoters are underlined. Dots indicate every tenth nucleotide. The Shine–Dalgarno sequence in the ribosome binding site and the start codon of the HGH mRNA are overlined. The *trp* repressor binding site (13) and the *lac* operator (11, 14) are indicated with broken lines. The transcription start sites are indicated with +1. In the case of *tac*I and *tac*II it is assumed that the transcription start sites are in the same region as those of the *lac* UV5 promoter (15). The fusion points between *trp* and *lac* sequences are at the open spaces in the *tac*I and the *tac*II sequences.

codes the Shine–Dalgarno sequence allowing protein synthesis to start at the ATG of the adjacent HGH gene.

Repression and Induction of the *tac*I Promoter and the *lac* UV5 Promoter. Fig. 3 shows the repression and inducibility of the *lac* UV5 promoter and the newly constructed hybrid promoter *tac*I. Cells of *E. coli* D12I0*lacI*^{q} containing the plasmid pHGH807*tac*I or pHGH107-11, which have the *tac*I or the *lac* UV5 promoter, respectively, were induced with 1.0 mM isopropyl β-D-thiogalactoside. The experiment shows that the *tac*I promoter can be repressed and induced effectively and that it is considerably more efficient than the *lac* UV5 promoter (see below).

Construction of *tac*II. We designed another hybrid *trp–lac* promoter (*tac*II), which we anticipated would also have an increased efficiency compared to the *lac* UV5 promoter. The *tac*II promoter was constructed in part from synthetic DNA. Its construction has been described elsewhere (11). The *tac*II sequence is shown in Fig. 2.

Measurement of the Relative Efficiencies of the Parental and the Hybrid Promoters. In order to compare the relative efficiencies of the promoters described here, we used plasmid pKM-1, a derivative of pKO-1, which has been designed for this purpose by Rosenberg and his colleagues (see refs. 7 and 12).

From the plasmids pHGH107-11, pHGH207-1, pHGH807*tac*I (Fig. 1), and pHGH907*tac*II (ref. 11) the small *Eco*RI fragments containing the *lac*, *trp*, *tac*I, and *tac*II promoters, respectively, were isolated and inserted into pKM-1 (see Fig. 4). Each plasmid was introduced in *E. coli* C600 *galK*$^-$ and *E. coli* HDB2 *galK*$^-$, *trpR*$^-$ and the galactokinase levels were determined.

The *lac* promoter and the hybrid promoters on pKM-1 are derepressed because the *lac* repressor in the host cells (*E. coli* C600) is titrated by the abundance of *lac* operator sequences on the high copy number plasmids (11, 14). In addition, any residual *lac* repressor activity was counteracted by the addition of 1 mM isopropyl β-D-thiogalactoside to the growth medium (16). The *trp* promoter on these plasmids is derepressed due to depletion of any residual tryptophan in the medium. The galactokinase levels in cells with pKM-1 harboring the various promoters are shown in Table 1. The galactokinase level in C600/pKM-*lac* is about 67 units/ml per OD_{650}. The galactokinase level in C600/pKM-*trp* is approximately 2.2 times higher than that in C600/pKM-*lac*UV5, implying that the *trp* promoter under these conditions is about 2 times more active than the *lac* UV5 promoter.

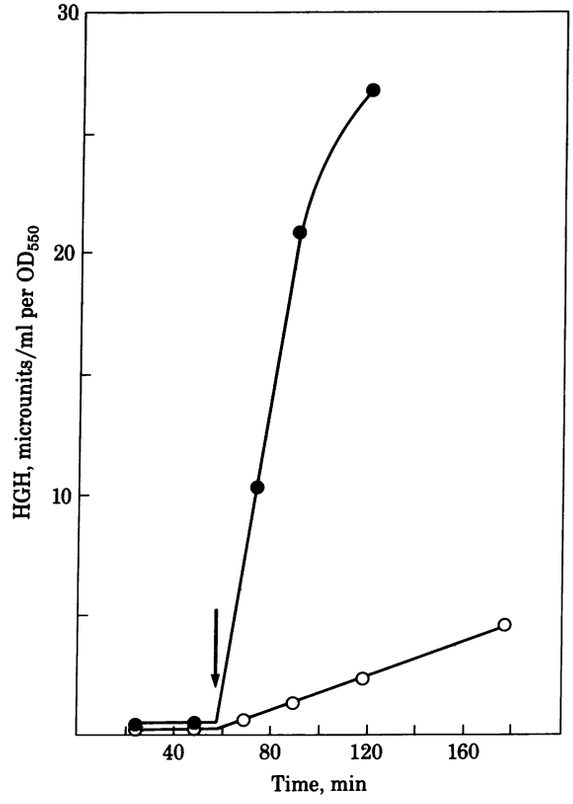

FIG. 3. HGH production directed by the *lac* UV5 promoter and the *tac*I promoter. Overnight cultures of *E. coli* D1210 were used to inoculate 50 ml of LB medium (16) containing ampicillin (20 μg/ml) to a cell density giving an OD_{550} of 0.03. At 60 min HGH production was induced with 1.0 mM isopropyl β-D-thiogalactoside. HGH levels were determined in a radioimmunoassay as outlined previously (8, 11). ○, *E. coli* D1210/pHGH107-11 (single *lac* promoter); ●, *E. coli* D1210/pHGH807*tac*I (*tac*I promoter).

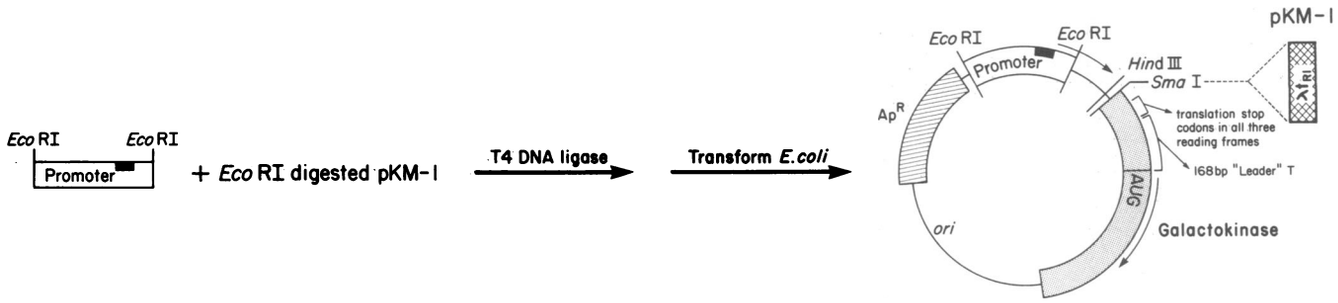

FIG. 4. Insertion of the *Eco*RI fragments containing the various promoters into the promoter probe plasmid pKM-1. This plasmid was obtained (7, 12) by insertion of a ρ-dependent terminator (λt_{RI}) into the *Sma* I site of pKO-1, which reduces the number of transcripts entering the *galK* gene to 30% or less (M. Rosenberg, personal communication; ref. 11). The promoter-containing pKM derivatives were used to transform either *E. coli* C600 *galK*$^-$ or HDB2 *galK*$^-$, *trpR*$^-$. Cells were plated on MacConkey galactose indicator plates. *ori*, Origin of replication.

The host used in this assay (*E. coli* C600) has a functional *trp* repressor. Previously (10) we found that the HGH levels in *trpR*$^+$ hosts are lower than those in *trpR*$^-$ hosts containing the plasmid pHGH207-1. It is likely that endogenously synthesized tryptophan causes some repression of the *trp* promoter. Hence, the relative *trp* promoter strength as measured in C600/pKM*trp* is underestimated. To determine the relative *trp* promoter strength accurately we constructed a derivative of *E. coli* C600 *galK*$^-$ that lacks a functional *trp* repressor (HDB2 *galK*$^-$, *trpR*$^-$). The pKM-1-derived plasmids containing the various promoters were introduced into HDB2.

The data in Table 1 show that the actual *trp* promoter strength as measured in HDB2/pKM*trp* is at least 3 times higher than that of the *lac* UV5 promoter. Table 1 also shows that the galactokinase level in HDB2/pKM-*trp* is about 1.6 times higher than that in C600/pKM*trp*. This increased *trp* promoter activity must be due to the absence of functional *trp* repressor in HDB2. No significant difference in promoter activity is observed in either host with the *lac* UV5 promoter.

The relative efficiencies of the hybrid promoters *tac*I and *tac*II were measured in a similar fashion in pKM-1 in both hosts. The *tac*I promoter appeared to be at least 10 times more efficient than the *lac* UV5 promoter and at least 3 times as strong as the fully derepressed *trp* promoter. The *tac*II promoter is about 7 times stronger than the *lac* UV5 promoter—i.e., the *tac*I promoter is 1.5 times stronger than the *tac*II promoter. The activities of the *tac*I and *tac*II promoters are not affected by the presence or absence of the *trp* repressor, which is consistent with the absence of an intact *trp* repressor binding site from both hybrid promoters.

DISCUSSION

In this paper we described the *in vivo* properties of two hybrid promoters. Both hybrid promoters can be repressed by the *lac* repressor and both can be derepressed by isopropyl β-D-thiogalactoside.

We showed that the *trp* promoter in the *trpR*$^-$ host is approximately 3 times stronger than the *lac* UV5 promoter. The *tac*I and the *tac*II promoters are about 11 and 7 times stronger, respectively, than the *lac* UV5 promoter. Thus the hybrid promoters are far more efficient than either one of the parental promoters. The differences between the hybrid promoters and each one of the parental promoters are too extensive to attribute the cause of this increased activity to one particular aspect of the hybrid promoter sequence.

The consensus sequence for the Pribnow box is 5′-T-A-T-A-A-T and that for the −35 sequence is 5′-T-T-G-A-C-A. Most promoter mutants map within the −35 or −10 area (reviewed in ref. 1). Almost all mutations that affect the promoter activity adversely decrease the extent of homology with the consensus sequence (1). The few mutations that increase promoter activity increase the extent of homology with the consensus −10 or −35 sequence (1). Thus it seems likely that promoters with a consensus −35 and a consensus −10 region and a distance of 17 bp (1, 2, 17) between these domains might be rather efficient. This also may hold true for the promoters analyzed here. The *lac* UV5 promoter has a consensus Pribnow box (T-A-T-A-A-T) but no consensus −35 sequence (T-T-T-A-C-A). The *trp* promoter does not have a consensus Pribnow box sequence (T-T-A-A-C-T) but it does have a consensus −35 sequence (T-T-G-A-C-A). Consequently, the *tac*I promoter not only has a con-

Table 1. Relative strengths of natural and hybrid promoters in pKM-1

Host	Plasmid/ promoter	Galactokinase units	Ratio relative to pKM-*lac*	Relative promoter activity		Galactokinase activity ratio, HDB2/C600	
				Promoters	Ratio	Promoter	Ratio
C600 *trpR*$^+$	pKM-*lac*	67	1.0	*tac*I/*tac*II	1.7 ± 0.2	*lac*	1.1 ± 0.1
C600 *trpR*$^+$	pKM-*trp*	144	2.2 ± 0.2	*tac*I/*trp*	3.5 ± 0.4	*trp*	1.6 ± 0.1
C600 *trpR*$^+$	pKM-*tac*I	796	11.8 ± 1.5	*tac*II/*trp*	2.1 ± 0.3	*tac*I	0.9 ± 0.2
C600 *trpR*$^+$	pKM-*tac*II	472	7.0 ± 0.9			*tac*II	1.2 ± 0.1
HDB2 *trpR*$^-$	pKM-*trp*	228	3.4 ± 0.5				

Cells were grown in M9 minimal medium (16) supplemented with 0.5% Casamino acids and 0.2% fructose to an OD_{650} of 0.6 and assayed for galactokinase activity as described (12). The galactokinase units are nmol of galactose phosphorylated per min per ml of cells at OD_{650} = 1.0. For each experiment two appropriate dilutions of the cultures were made and the galactokinase activity was determined in triplicate. The average value was taken of the dilutions for which the total radioactivity incorporated into galactose phosphate was less than 25% of the input radioactivity. The results shown are the mean values (±SEM) of eight or more independent experiments. The relative strengths of the hybrid promoters and the *trp* promoter are shown in column 6; results are the mean (±SEM) of eight such ratios of eight independent experiments. The ratios of the galactokinase activities in HDB2 and C600 harboring the various promoters in pKM-1 are given in the last column. The mean (±SEM) of five independent measurements is shown.

sensus −35 sequence (T-T-G-A-C-A) but also has a consensus Pribnow box sequence (T-A-T-A-A-T). In *tac*I the distance between both domains is 16 bp which, by comparison with the mutant *lac* p^s promoter (17), may be suboptimal.

The *tac*II promoter has a consensus −35 sequence 5′-T-T-G-A-C-A but no consensus Pribnow box sequence (T-T-T-A-A-T). The distance between the two domains in *tac*II is 17 bp. It is possible that, in this promoter, the optimal distance compensates for a suboptimal Pribnow box sequence, resulting in a promoter that is much stronger than the *lac* UV5 promoter.

We realize that the increased efficiency of the hybrid promoters compared to the parental *lac* UV5 promoter may be merely due to an optimization of the distance between the two domains from 18 bp in the parental *lac* UV5 promoter to 17 bp and 16 bp in the *tac*II and *tac*I promoter, respectively.

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