A convenient technique to compare the efficiency of promoters in *Escherichia coli*

D. Vidal-Inigliardi and O. Raibaud

Unité de Génétique Moléculaire, Institut Pasteur, 28 rue du Dr. Roux, 75724 Paris Cedex 15, France

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**ABSTRACT**

We describe a technique which allows one to insert any promoter in front of the chromosomal malPQ operon. This can be done easily by using only one plasmid, one strain, and two simple selections. Properties of the final chromosomal fusion are such that the level of amylomaltase, the product of the malQ gene, measures quantitatively the efficiency of the inserted promoter. This method was utilized to compare the efficiency of four well-known promoters: lacZp, trp, tac, λPR and three malT activated promoters: malPp, malKp and malEp.

**INTRODUCTION**

The control of expression of prokaryotic genes occurs primarily at the level of the initiation of transcription. It is therefore important to have an easy and reliable procedure to compare the activity of bacterial promoters in vivo. A widely used approach is to fuse different promoters to the same gene, the product of which is easily assayed. A major limitation of this approach is that the translation efficiency of this gene may be altered in the hybrid transcription unit. In the technique of McKenney et al. (1) where the promoters are fused to the galK gene, the problem of translation efficiency was overcome. In that case, the genetic constructions were performed on a multiple copy plasmid, and this introduced a certain degree of unreliability in the quantitative comparison of promoter strengths. Indeed the stability of the plasmid was affected when it harboured a strong promoter (2,3), the copy number of the plasmid was subject to variability depending on the inserted promoter and on the growth conditions (2), and the presence of multiple copies of a promoter could lead to a titration of regulatory proteins controlling its activity (4,5). A procedure to transfer the above genetic constructions from the multiple copy plasmid to the bacterial chromosome was described (1), but it is rather laborious and has not been used extensively.
In this paper we describe a more convenient technique to compare the efficiency of bacterial promoters. It allows one to transfer any promoter which is active in E. coli onto the chromosome of this bacterium, in front of the malPQ operon, such that the activity of the promoter can be determined by assaying amylomaltase, the product of the malQ gene. As an application, we have compared the efficiency of promoters which are widely used in expression vectors, namely lacZp, trp, tac, and λPR.

MATERIALS AND METHODS
All of the methods used in this work were described previously (6). The DNA fragments carrying the promoters are schematically represented in Fig.1 and were obtained as follows.

The lacZp promoter: A 207 bp EcoRI fragment containing this promoter was obtained as previously described (7).

The trp promoter: A 76 bp EcoRI fragment containing the trp promoter was purified from plasmid pDR720 (8).

The tac promoter: The ptac12 plasmid (9) was linearized with PvuII and religated in the presence of an excess of EcoRI linkers. A 273 bp EcoRI fragment containing the tac promoter was purified from the plasmid thus obtained.

The λPR promoter: A 2391 bp BgIII fragment, containing the PR promoter as well as the CI gene, was obtained from the λ CI857 phage and cloned into the BamHI site of pSB118. This plasmid, constructed by P.Stragier, is a derivative of pUC18 (10), in which the polylinker is flanked by two EcoRI sites. A 2451 bp EcoRI fragment, containing the PR promoter and the CI857 gene, was obtained from the recombinant plasmid.

The malKp and malEp promoters: A 418 bp TaqI fragment containing these two promoters, was purified from pSF630 (11) and inserted into the AccI site of pSB118. A 478 bp EcoRI fragment carrying the malEp and malKp promoters was purified from the recombinant plasmid.

RESULTS -DISCUSSION
Insertion of any promoter containing fragment in front of the chromosomal malPQ operon.

In a previous paper we described pOM40, a plasmid vector which permits the insertion of any DNA fragment into the chromosome of E. coli. pOM40 is a derivative of pBR322 containing a 6 kb insert including malPp, the promoter of the malPQ operon. In this plasmid the tet gene is controlled by malPp.
A derivative of pOM40, called pOM41, has now been constructed which is better adapted to the cloning of promoters. It is identical to pOM40 with the exception that a DNA segment extending from positions -34 to -155 with respect to the transcription start point in malPp has been eliminated. The effect of this deletion (malPp\(\Delta\)34) has been to inactivate malPp, such that the tet gene is no longer expressed in the plasmid (Fig. 2). This offers two advantages. The first is that it is now possible to use tetracycline resistance as a means to select for plasmids into which a promoter containing fragment has been inserted. The second is that the deletion has eliminated the binding sites for two activator proteins, MalT and CAP, which are normally present at the malPp promoter (12) and which could interfere with the functioning of foreign promoters inserted in the EcoRI site.

The transfer of a promoter in front of malPQ thus can be performed as described in Fig. 2. The promoter is first cloned in pOM41, by selecting for Tet\(^R\) transformants. It is then transferred onto the chromosome, in place of AmalA510, by selecting for Mal\(^+\) recombinants. Finally plasmid free recombinants (Amp\(^S\)) are obtained by reisolation of colonies in the absence of ampicillin, and the activity of the promoter is determined by assaying amylomaltase, the product of the malQ gene.

Is the level of amylomaltase proportional to the activity of the inserted promoter?

In principle, the above procedure makes it possible to compare the efficiency of any desired promoter by placing it in front of the malPQ operon and assaying amylomaltase. Since the promoters are inserted in the
Figure 2. Transfer of a promoter containing fragment in front of the malPQ operon.

Plasmid pOM41 is essentially pBR322 with an insert containing gene malT and the beginning of the malPQ operon (6). The brackets correspond to a 120 bp deletion (malPPΔ534) which inactivated the malPp promoter, and the filled arrowhead indicates the position of the unique EcoRI site present on the plasmid. The EcoRI fragment containing the foreign promoter (open arrowhead) is cloned into the EcoRI site of pOM41, by selecting for plasmids which confer resistance to tetracycline. The fragment is then transferred onto the chromosome, in place of deletion ΔmalA510, by selecting for Mal+ recombinants. On the last line is shown the nucleotide sequence separating the EcoRI site from the initiation codon of malP. Nonsense codons (n.s.) are shown, as well as the Shine and Dalgarno sequence (S.D.) and the initiation codon of malP (met).

chromosome as a single copy, the complications due to the use of multiple copy plasmids are eliminated. However, the assumption that the level of amylocyclase synthesis faithfully reflects the activity of the inserted promoter needs to be discussed. Three possible reasons why this might not be the case can be considered. The fusion of the foreign promoter to the malPQ operon (i) could create a transcription termination signal between the promoter and the malQ gene, (ii) it could affect the stability of the malQ mRNA and, (iii) it could change the efficiency of translation of the malQ gene. These three possibilities apply to all systems of gene fusion where one measures promoter efficiency by assaying a translation product. Regarding the first point no more than 100 nucleotides at the 5'-end of the mRNA were untranslated in all the constructions we have done. This limited
seriously the risk of creating a rho dependent termination site in this part of the DNA (13). In addition sequencing data show that there are no rho independent termination site in these regions. A more difficult problem concerns mRNA stability, which may depend on the structure of its 5'-end. Although the mechanisms of mRNA degradation are still controversial (14, 15, 16), we think it is unlikely that small differences at the 5'-end, 2500 nucleotides upstream of malQ, should have a drastic effect on the stability of malQ messenger itself. Finally the possible effects of the fusion event on malQ translation should be minimal for two reasons. First, the sequence between the EcoRI site and the initiation codon of malP contains a nonsense codon in the three phases (Fig.2). Therefore translation events initiated in the cloned fragment should not interfere with the initiation of translation at the beginning of malP. Secondly, even though it is possible that differences in the structure of the mRNA at its 5'-end may affect the initiation of malP translation, these differences should have very little influence on malQ translation. Previous work has indeed shown that a reduction of malP translation by a factor of 25 decreases malQ expression by a factor of only 2 (17).


Using the above procedure we determined the activity of seven promoters which had been extensively studied in vivo and, for some of them, in vitro. (Table 1). A few comments can be made on the results. One is that the activities of the different promoters are all of the same order of magnitude, differing by a factor of 5 or 6 at most. In this respect it is interesting that the tac promoter, often considered very strong, and stronger in particular than the "parental" promoters trp and lacZp (9), is in fact slightly less active than trp, and only twice as active as the lac promoter. Another comment concerns the mal promoters. The malEp, malKp and malPp promoters, all controlled by a single activator protein, MalT, are very different structurally (12). Two of them, malPp and malKp, seem to have three binding sites for the MalT protein, whereas malEp has only one. In addition the malEp and malKp promoters are CAP and 3'-5' cyclic AMP dependent, whereas malPp is not. In spite of these differences the three mal promoters are shown here to have very similar activities and induction ratios.

Four of the promoters, trp, λPR, malEp and malKp were found to be regulated in the same way when present in front of malPQ as when they are at their normal locations. In contrast, the activity of the lacZp promoter is thirty
Table 1. Efficiency of several promoters, as measured by *malQ* expression

<table>
<thead>
<tr>
<th>Strains</th>
<th>Relevant genotype</th>
<th>Amylomaltase (u/mg)</th>
<th>Induction factor</th>
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</thead>
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<tr>
<td></td>
<td></td>
<td>Uninduced</td>
<td>Induced</td>
</tr>
<tr>
<td>pop2308</td>
<td>malPp::534</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>pop2309</td>
<td>malPp::534::lacZp</td>
<td>-</td>
<td>510</td>
</tr>
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<td>malPp::534::trp</td>
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<td>1410</td>
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<td>-</td>
<td>950</td>
</tr>
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<td>265</td>
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<td>250</td>
</tr>
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<td>10</td>
<td>390</td>
</tr>
<tr>
<td>pop2329</td>
<td>malPp::534::tac</td>
<td>260</td>
<td>1100</td>
</tr>
</tbody>
</table>

(a) All strains are derived from pop3, our designation for strain MC4100 (F- araD139 ΔlacU169 thiA rpsL relA flaB) (18) except pop2328 and pop 2329 which are derived from Hfr66 (Hfrhis) (19). Pop2328 and pop2329, unlike strains derived from MC4100, contain an intact lacI gene. They were obtained by transducing with phage P1 the malA region of pop2309 and pop2313, respectively, into pop2170 (Hfr66 Δ malA510) (20). Strain pop2316 was obtained by selecting spontaneous Mal+ clones from pop2311 at 30°C, and verifying by complementation that the mutation was indeed in the CI gene.

(b) Amylomaltase was assayed as previously described (12). All values are the average obtained from at least three independent cultures.

(c) All strains were grown at 37°C (except for pop2311 which was grown at 30°C (uninduced) or 42°C (induced)), in M63 minimal medium containing 0.4% glycerol as a carbon source. Induction was obtained by growing the cells for four generations in the following conditions: for lacZp and tac, addition of 1mM Isopropyl-thio-β-D-galactoside; for trp, addition of 5μg/ml 3-β-indolacrylic acid (8); for malEp, malKp and malPp, addition of 0.4% maltose.

(d) The induction factors shown in the last column are from the references given in parenthesis.

Times less repressed by the lac repressor when present in front of *malPQ*. The tac promoter is even more weakly repressed by the lac repressor, even though the lac operator is correctly positioned with respect to the
transcription startpoint. This result, and a similar observation recently made with the mac promoters, which are hybrids between the malPp and lacZp promoters (26), provide a strong indication that sequences other than the lac operator are required for full repression by the lac repressor.

CONCLUSIONS
The procedure which is described herein allows for the quantitative comparison of the efficiency of promoters in vivo. This constitutes a useful complement to in vitro studies on transcription initiation (27). The various parameters used in the in vitro experiments, such as the concentration of RNA polymerase or the degree of DNA supercoiling, are usually chosen rather arbitrarily. By comparing the in vivo and in vitro data, it should be possible to adjust these parameters so that the hierarchy of promoters efficiency would be the same in vivo as in vitro (27, 28).

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