Effect of rifampicin on expression of lacZ fused to promoters or terminators of the E.coli rpoBC operon

Kathy M. Howe, Andrew J. Newman, Ian Garner, Anne Wallis and Richard S. Hayward

Department of Molecular Biology, University of Edinburgh, Edinburgh EH9 3JR, UK

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ABSTRACT

The genes encoding the β and β' subunits of RNA polymerase in E.coli lie downstream of at least two ribosomal protein genes in a single unit of transcription. Treatment of E.coli with rifampicin, under conditions producing partial inhibition of general RNA synthesis, can strongly stimulate transcription of the polymerase genes, while activating the neighbouring ribosomal genes only slightly. We have investigated the mechanism of this effect by fusing strong promoters, a weak internal promoter, and an attenuator of the polymerase operon to the lacZ gene, in derivatives of plasmid pMC81. Studies of these fusions confirm our conclusion, based on similar fusions to galK, that rifampicin can foster readthrough of transcriptional terminators. They also suggest the existence of extra terminators and anti-termination elements in the above transcription unit.

INTRODUCTION

The structure of the rpoBC operon of E.coli, shown in Fig. 1, is such that the rpo genes which encode the β and β' subunits of RNA polymerase lie downstream of, and are obligatorily co-transcribed with several ribosomal protein (rpl) genes: see reviews by Yura and Ishihama (3) and Matzura (4), briefly updated in (5). However, certain growth constraints can partially uncouple the transcription of the rpo and rpl genes (reviewed in 3, 4, 6, 7). In particular, when general RNA synthesis in E.coli is partially blocked by the inhibitor of initiation, rifampicin, rpoBC-transcription is paradoxically stimulated, while transcription of the rpl genes is only weakly affected (8, and other papers cited in 5). We have attempted to define the transcriptional signal(s) mediating stimulation of rpoBC expression by rifampicin, by fusing the strong shared promoters of the rplKAJL and rpo genes (P_{L11} and/or P_{L10}) to lacZ in plasmids derived from pMC81 (9). We have similarly fused to lacZ the weak promoter (P_{β'}) and the partial terminator or "attenuator" (tL) which lie between the rpl and rpo genes in the rpoBC operon. We have then examined the effects of low concentrations of rifampicin on expression of lacZ by strains harbouring the fusion plasmids. Our results support the
Fig. 1 Maps (to scale) showing the genes, transcriptional signals, protein products, and targets for relevant restriction enzymes, in DNA of \textit{A}rifd18 carrying (A) the rplKAJLrpoBC operon and (B), in more detail, the EcoRI fragment spanning the rplL-rpoB junction; derived principally from Post et al. (20); see also 21, 22. No BamHI targets are present. The function of the 20 Kd U-gene product is unknown. The coordinates shown in map A are in kilobases, on the scale of Post et al. (20); fragment sizes in B are in base-pairs. : map position of transcription signal (tentative for P, and P_R1); <X>: strong promoter; +: weak promoter; -<X>: partial terminator; X: strong terminator.

conclusion, derived from studies of similar fusions to \textit{galK} (described in the accompanying paper, ref. 5) that rifampicin can decrease the response of RNA polymerase to many transcriptional terminators, including rpop1. They also show that P_B is not stimulated by rifampicin, and that streptolydigin does not mimic the latter drug in its effects on termination. Finally they suggest the possible existence of extra terminators and anti-termination elements in the rplKAJLrpoBC operon.

MATERIALS AND METHODS.

Strains. The strains of E.coli K12 used were ED8654 (\textit{hisD} \textit{hisM} supF supF) from Dr. N.E. Murray, ED8641 (\textit{recA} \textit{trpE} \textit{trpE} \textit{trpR}) from Prof. W.J. Brammar, and MC1000 (\textit{araD} \textit{ara-leu} \textit{galU} \textit{galK} strA) from Dr. M.J. Casadaban (9). The plasmid vector used, was pMC81, also from Dr. Casadaban (9). \textit{A}rifd18 is a defective transducing phage carrying the \textit{rrnB}-rpoC region of the E.coli K12 chromosome, including a dominant
Rif-R allele of rpoB (10, 11). λAJN81 is a derivative of λ590 (12), carrying the 335 bp Alul fragment of the rpoBC operon which includes the t1 and $P_\beta$ transcription signals (Fig. 1). It was constructed (A.J.N.) using HindIII molecular linkers, as described in the accompanying paper (5). Phage, plasmid and DNA manipulations were carried out as cited previously (5).

β-galactosidase assays followed Miller (13), with some modifications noted below. Cultures of E.coli MC1000 harbouring the appropriate pHCl81-derivative were grown overnight, 37°C, 200 r.p.m. rotation in minimal medium (Spizizen salts, glycerol (0.2%), casamino acids (0.1%), ampicillin (50 μg/ml)) with the addition, where indicated, of arabinose as a non-metabolisable inducer of lacZ in strains carrying the $P_\text{ara}$-lacZ fusion (9). Doubling times in the absence of arabinose were 50 (+5) min; addition of arabinose increased these doubling times to 63(±3) min for most strains (96(±9) in the case of the parent plasmid). The degree of this effect appears to be correlated with the extent of over-synthesis of products of genes fused to $P_{\text{ara}}$. Enzyme assays were initiated after a 50-fold dilution of an overnight culture, in the same medium, had grown back to an A$_{600}$ between 0.28 and 0.35. 1 ml samples of the cultures (diluted if necessary with fresh medium) were mixed with 0.8 ml of PM2 buffer (120 mM sodium phosphate, pH7.0; 1 mM MgSO$_4$; 0.2 mM MnSO$_4$; 10 mM 2-mercaptoethanol), 0.2 ml of 0.1% cetyl trimethyl ammonium bromide, and 10 μl of fresh 1% sodium deoxycholate. After vortex-mixing the samples could if necessary be stored for up to 30 min on ice, then brought to 28°C for 5 min before addition of 0.6 ml of 13.5 mM o-nitrophenyl-β-D-galactopyranoside, and mixing, to initiate the reaction. Reactions were terminated with 1.3 ml of 1M Na$_2$CO$_3$. Spectrophotometric assay, calculations, and controls are described by Miller (13).

Sources of materials were as described previously (5), with o-nitrophenyl β-D-galactopyranoside from Sigma.

RESULTS

Fusion of rpo elements to lacZ. In order to assess the role of different transcriptional signals of the rpoBC operon in rifampicin-induction of rpoBC expression, we fused them separately to lacZ in vitro, exploiting the vector pMC81 (9) whose structure is summarised in Fig. 2. The easily assayable β-galactosidase enzyme is encoded by lacZ, which is here served by the arabinose-inducible $P_{\text{ara}}$ promoter (araI; accompanied by its regulator gene, araC). Foreign DNA can readily be inserted at the unique KpnI or HindIII
Fig. 2. Maps (to scale) of the vector pMC81 (9) and of our derivatives containing inserted DNA from the rpoBC region of λrifd18. Only the restriction targets relevant for construction or characterisation of our fusion plasmids are shown on each map (with their coordinates in kilobases; all plasmids are drawn as if linearised at a particular BamHI site). Relevant genes, transcriptional signals, and normal transcriptional orientations (horizontal arrows) are also shown. araC.OP represents the operator and promoter of the E.coli ara operon, accompanied by their repressor-/+ activator-protein gene (araC); see (9) and Fig. 1 for other gene symbols. Bam: BamHI-restriction target; Kpn: KpnI-; HII: HindIII-; RI: EcoRI-; Sma: SmaI-; Sal: SalI- (note that pMC81 and pHR6 contain no SalI-targets); P: PstI-; *: BamHI/BglII fusion-site. We have verified all the restriction maps shown.

pHR5 is identical to pHR4, but has the opposite orientation of the "tl" insert (SalI-site at 6.32 Kb). pIG103 resembles pHR7, but the DNA between the HindIII sites carries the terminator tec3 from coliphage T7 (ref. 5) in its functioning orientation, on a 179 bp Alul-fragment provided with HindIII-molecular linkers.

restriction targets, which lie between P ara and lacZ. It should be noted that the HindIII cloning site lies within trpB; therefore inserted DNA may generate strong polar effects on lacZ expression, whether or not it contains transcriptional terminators (as demonstrated by Barry et al (14). Polarity is due to translational effects in this case (C. Squires, pers. comm.). In pMC81, trpA' and lacZ are fused in phase, such as to generate a functional β-galactosidase. Although the enzyme is almost normal in activity, its
synthesis is dependent on initiation at the trpA start. Since trpA translation is coupled to that of trpB (cf. 29), interruption of the latter by frameshifting insertions at the HindIII site severely depresses translation of lacZ-mRNA derived from pMC81.)

A 335 bp Alul-fragment carries the "carboxy-terminal" 23 nucleotides of rplL, and most of the rplL-rpoB intercistronic region, including tl and the presumptive PB; it ends only 12 nucleotides short of rpoB (Fig. 1). This fragment was ligated to HindIII linkers and cloned in λ590 to generate phage λAJN81 (5). The 345 bp HindIII fragment was then transferred into the corresponding site of pMC81, using a screening procedure based on insertional inactivation of the trpB gene. trpB is intact in pMC81, and can be expressed from P ara (araI) if this is activated by arabinose (9). Strain ED8641 is (trp E - B)A and trpR; it is auxotrophic for tryptophan and, being trpBA, is also unable to grow on indole. ED8641 harbouring pMC81 can grow on indole if arabinose is provided.

The DNA of pMC81 and λAJN81 were digested with HindIII, mixed, ligated, and used to transform ED8641 to ampicillin-resistance. Individual transformants were transferred to minimal Spizizen-arabinose-glycerol-acid casein hydrolysate-indole (5 μg/ml) plates with and without tryptophan (20 μg/ml). Analysis of plasmid DNA from Amp-R, Ind- transformants, by restriction with HindIII, EcoRI and SalI endonucleases, identified plasmids having the rpoI fragment inserted in either orientation (Fig. 2). In pHR4, tl is oriented such that before reaching lacZ, RNA polymerase initiating at P ara will traverse the terminator as it would in the natural rpoBC operon. pHR5 has the opposite orientation of the HindIII insert. For the subsequent studies pHR4 and -5 were transferred into E.coli MC1000.

To fuse lacZ to the major rpoB promoter, P10, the 1.6 Kb BamHI-HindIII fragment of pMC81 (Fig. 2) was replaced by the 1.25 Kb BglII-HindIII ('rplA-rplJ') fragment of λrifd18 (Fig. 1). The resulting plasmid, pHR6, rendered E.coli MC1000 Amp-R, and Lac⁺ in the absence of arabinose. Restriction analyses of pHR6 confirmed that its structure is as shown in Fig. 2 (e.g. a BamHI site has been destroyed, and a PstI introduced). Thus the RNA polymerase-binding portion of P ara should have been removed (Greenfield et al; 15), and lacZ expression made dependent upon P10. Another pMC81 derivative, having both P11 and P10 fused to lacZ in the correct orientation for transcription, was constructed by replacing the 0.8 Kb KpnI-HindIII fragment of pMC81 (Fig. 2) with the 2.6 Kb (KpnI)-'U-P11-rplKA-P10-rplJ'-(HindIII) fragment of λrifd18 DNA (Fig. 1).
The resulting plasmid, (pHR1), has the structure shown in Fig. 2, as confirmed by restriction analysis (e.g. the replacement introduced one new EcoRI and five new PstI sites).

The HindIII fragment bearing rpotI was introduced from λAIJN81 into pHR1 by ligation with pHR1 cut with HindIII and calf intestinal phosphatase. Among the products was pHR7, which has rpotI inserted in its normal orientation between the rpl promoters and lacZ (Fig. 2). The HindIII fragment carrying a newly discovered terminator from the late region of coliphage T7 DNA, T7 tec3 (5) was similarly transferred into pHR1, in its functioning orientation, to generate plasmid pIG103.

The sketches in Fig. 2 summarise the regulatory signals which can be expected to affect lacZ expression in the various plasmids.

Expression of lacZ by the novel fusions. The steady state levels of β-galactosidase activity in MC1000 harbouring pMC81 and its rpo fusion derivatives were determined during exponential growth (Table 1). L-arabinose, when present continuously or freshly added as a non-metabolisable inducer of P ara, was at a final concentration of 0.1%.

The data obtained without arabinose confirm that P T1 is serving lacZ in pHR6, and indicate that, surprisingly, the addition of P L11 (in pHR1) provides little or no extra lacZ transcription. (Note that comparison of pHR1 and pHR6 should not be invalidated by polar effects of the fusions upon lacZ expression; the downstream fusion junctions are identical, so polarity effects should be equal).

The data obtained with arabinose-induced cultures show that the fragment carrying the terminator t1 decreases lacZ expression very strongly when inserted into pMC81 either in the normal (pHR4) or the inverse (pHR5) orientation. Termination in both orientations was also observed in fusions between P gal and galK (5), although the effect of the inverted fragment was weaker in that case (73% cutdown). Polarity may be contributing an extra effect in the lacZ fusion; it is minimised in the galK fusions. Interestingly, the rpotI DNA may cause less efficient termination when fused to P L11/P L10 than to P ara: compare 88% cutdown for pHR7 with 98% for pHR4 (Table 1). This will be discussed later.

Despite the presence of P ara on pHR1, arabinose barely induces increased synthesis of β-galactosidase in MC1000 (pHR1) (or MC1000 (pHR7)): contrast the pMC81 derivative. A plausible explanation is the existence of one or more strong terminators of transcription downstream of P ara in pHR1. (These results also suggest that transcription initiated at P ara could contribute
<table>
<thead>
<tr>
<th>Plasmids harboured by E. coli MC1000</th>
<th>Known promoters and terminators affecting lacZ</th>
<th>Units of β-galactosidase (per A₆₀₀)</th>
<th>Extent of cutdown (%)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>−ara</td>
<td>+ara</td>
</tr>
<tr>
<td>pMC81</td>
<td>ara</td>
<td>529 (±23)</td>
<td>11860 (±660)</td>
</tr>
<tr>
<td>pHR4</td>
<td>ara → t1 Pᵦ</td>
<td>10 (±1)</td>
<td>222 (±24)</td>
</tr>
<tr>
<td>pHR5</td>
<td>ara ← t1 Pᵦ</td>
<td>15 (±3)</td>
<td>417 (±18)</td>
</tr>
<tr>
<td>pHR1</td>
<td>ara Pₐ₁₁ Pₐ₁₀</td>
<td>427 (±17)</td>
<td>459 (±3)</td>
</tr>
<tr>
<td>pHR7</td>
<td>ara Pₐ₁₁ Pₐ₁₀ → t₁ Pᵦ</td>
<td>45 (±1)</td>
<td>56 (±2)</td>
</tr>
<tr>
<td>pIG103</td>
<td>ara Pₐ₁₁ Pₐ₁₀ T₇ tec3</td>
<td>126 (±3)</td>
<td>−</td>
</tr>
<tr>
<td>pHR6</td>
<td>Pₐ₁₀</td>
<td>390 (±16)</td>
<td>−</td>
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The figures are units of enzyme activity (13) for cultures grown and assayed as described in Methods, with the standard errors of their means given in brackets. The means were derived from a minimum of eight separate assays, using at least two independent cultures of each strain (except pMC81 and pIG103 −ara, and pHR7 +ara; single cultures). "+ara": grown in continuous presence of arabinose.

The superscript arrows show (where known) whether the terminator insert is orientated with respect to transcription as in its normal environment (→), or with the opposite sense (←). T₇ tec3 is in its functioning orientation in pIG103; its physiological status in T₇ is unknown.

"Plateau" activities attained 40–70 min after addition of arabinose (0.1%) to exponential cultures (A₆₀₀ = 0.4).

Reduction of enzyme activity ascribable to the terminator insert.
very little of the β-galactosidase-mRNA produced by pHRl in the absence of arabinose).

Variations in plasmid copy-number per cell might in principle affect the results shown in Table 1. However, small-scale preparations of plasmid DNA analysed on agarose gels revealed no detectable differences in plasmid yields from the various strains.

**Effect of rifampicin on lacZ expression.** The effects of rifampicin addition (10 μg per ml) on β-galactosidase synthesis directed by the fusion plasmids are illustrated in Fig. 3. Here the changes in activity (per A₆₀₀, i.e. corrected for increases in culture density) are shown as a function of time after drug addition. The level of drug used had no significant effect on growth (A₆₀₀) over the 50 min duration of these experiments.

Rifampicin has no detectable effect on β-galactosidase synthesis in the cases of pMC81 (Fig. 3A) or pHR5 (Fig. 3C; but see below). A modest stimulation is observed when rpoT1 is present in the "normal" orientation downstream of ParA (pHR4: Fig. 3B); but dramatic stimulations (with a noticeable lag) occur for pHRl, grown with or without arabinose (Fig. 3D and E), and especially for its derivatives carrying the rpoT1 and T7 tec3 terminator inserts, pHR7 (Fig. 3F) and pIG103 (3G). A much weaker stimulation (Fig. 3H) is observed with pHR6, which like pHR1 carries PL₁₀ and has the same downstream DNA fusion, but lacks additional DNA (ParA-arab'-Mu'-U-P₉₁₁-rplKA') present upstream in pHR1.

Other experiments (not shown) tested the effects of rifampicin (20 μg/ml) on induction of β-galactosidase in log-phase cultures, by addition of arabinose with or without the drug. In the control, MC1000 (pMC81), the presence of rifampicin inhibits accumulation of enzyme, as expected (by 40% at 10 min after arabinose addition, and 75% after 50 min). In contrast the drug at first stimulates induction in MC1000 (pHR4)—by 30% at 10 min—although initiation at ParA is presumably being inhibited just as in pMC81. (Inhibition occurs only after 60 min, for pHR4). We assume that the early stimulation is due to the presence of rpoT1 in pHR4. pHR5 (with the rpoT1 fragment inverted) shows no absolute stimulation by rifampicin, but the overall inhibition is markedly less than for pMC81. It is noticeable that the drug effects on the lacZ fusions of pHR4 (and pHR5) are weaker than for the corresponding galK fusions (5). Perhaps this is due to the different promoters operating in the two cases, or to translational differences (cf. Table 2 footnotes).

Similar experiments established that, in contrast to rifampicin, streptomycin (80 μg per ml; growth (A₆₀₀) unaffected during course of experiment).
Fig. 3. Effect of rifampicin addition (10 μg per ml) on β-galactosidase accumulation in E. coli MC1000 harbouring pMC81 and various derivatives. Activity (units/A600) is plotted as a percentage of the pre-drug level, versus time after drug addition. ○: no drug; ●: with drug. Vertical bars indicate the standard errors of the means (where these are greater than the symbol-diameters). All means are derived from 4 to 8 separate assays (2 to 4 separate cultures), except for C, E, and G (duplicate assays from single cultures). The plasmids, and the known transcriptional signals affecting lacZ expression in each case are as follows: A, pMC81 (P ara, activated); B, pHR4 (P ara (activated) followed by rpoT1 terminator); C, pHR5 (P ara (activated) followed by the rpoT1-DNA inverted); D, pHR1 (P ara (activated) followed by P L11 and P L10); E, pHR1 (P L11 and P L10); F, pHR7 (P L11 and P L10 followed by rpoT1); G, pIG103 (P L11 and P L10 followed by T7 tec3); H, pHR6 (P L10). P ara was activated, in A to D, by the continuous presence of 0.1% arabinose. Note the different ordinate scale used in frames F to H.

Inhibits induction of lacZ as strongly in MC1000 (pHR4) as in MC1000 (pMC81): data not shown. Note that streptolydigin, unlike rifampicin, fails to induce increased ββ' synthesis in E. coli (16). Finally, rifampicin causes no detectable induction of β-galactosidase synthesis by MC1000 (pHR4) in the absence
of arabinose. We conclude from this that the weak rpo promoter P_\text{fi}, which is fused to lacZ in pHRA, is not significantly activated by rifampicin.

**DISCUSSION**

Rifampicin, under conditions producing partial inhibition of general RNA synthesis in E.coli, stimulates rpoBC-transcription strongly, and rplKAJL-transcription slightly (8). The results reported here appear to exclude one possible explanation of the former effect; the weak P_\beta promoter, when fused to lacZ, is not detectably activated by rifampicin. (Although we have not examined possible activation of the weak P_{L1Q} promoter experimentally, it can probably be excluded on theoretical grounds. Its activation should lead to stimulation of L7/12 as well as \beta and \beta' synthesis, but this is not observed (17). The failure to stimulate L7/12 synthesis cannot be ascribed to auto-repression of its translation, since L7/12 does not display this property (18).)

The most obvious remaining explanation of the transcriptional stimulation of rpoBC (8) is that rifampicin increases readthrough of the terminator t1; but because the probes used by Blumenthal and Dennis (8) did not distinguish rplKA from rplJL transcription, stimulation of the P_{L1Q} promoter is an additional or alternative possibility. The present studies (Fig. 3) clearly suggest that rifampicin (10 \mu g/ml) does cause readthrough of rpotl and of another terminator, T7 tec3, when these are inserted upstream of lacZ in plasmids harboured by rpoB^+ E.coli. Fusions to galK have also suggested that both these and three other terminators are rendered less efficient in the presence of the drug. We have reported those results elsewhere, have argued that all are very likely to reflect transcriptional effects (although this remains to be proved by mRNA analysis), and have concluded that low concentrations of rifampicin increase readthrough of transcriptional terminators generally (5). The results of both studies favour the view that rifampicin stimulates rpoBC transcription mainly by increasing readthrough of rpotl. The present work has also shown that streptolydigin does not have this effect, consistent with our previous observation (16) that it does not stimulate chromosomal rpoBC gene expression in E.coli. The question, whether or not rifampicin has a special effect at rpotl additional to that observed for other terminators, is discussed elsewhere (5).

Can the remaining rifampicin-stimulations observed in Fig. 3 be explained in the same way? Could drug-stimulation be diagnostic for transcriptional termination between a promoter and the gene monitored? Considering
first the case of pHRl, the absence of significant lacZ induction by arabinose (Table 1) already suggested the existence of one or more terminators between P ara and lacZ. To explain the observed strong drug-stimulation of lacZ expression by pHRl in the absence of arabinose, we would then require that one or more of the above terminators lies between P \text{L11} and P \text{L10} (cf. Fig. 1). (Termination downstream of P \text{L10} cannot be invoked, since pHR6 shows only a weak stimulation by rifampicin). The results of Brückner and Matzura (30; also C. Squires, pers. commun.) suggest that termination between rplA and P \text{L10} is quite rare. However, the possible existence of a terminator some 40 nucleotides downstream of P \text{L11} was suggested by Post et al (20). We therefore suggest that the strong effect of rifampicin on lacZ expression by pHRl is mediated by a terminator immediately downstream of P \text{L11}.

It should be noted that the rifampicin effects observed for pHRl are unlikely to be due to polarity mechanisms. Any polarity effects in pHRl and pHR6 should be identical (same fusion), but the drug effect on pHR6 is much weaker than for pHRl. Moreover, in the mRNA generated by both plasmids ribosomes should read from rplJ into trpB in phase, so that polarity arising from premature termination of translation (23) is not expected.

The weak rifampicin-stimulation of lacZ expression by pHR6 might in principle be ascribed to a direct stimulation of the promoter P \text{L10}', but could be explained by the general model if there is a weakly affected transcriptional terminator between P \text{L10} and rplJ. There is published evidence of either termination or processing of RNA some 250 nucleotides downstream of P \text{L10} (20, 30). Note, on the other hand, that a fusion of P \text{L10} rplJ to galK showed no detectable drug effect (pHR8 in ref. 5).

Whatever the explanation of the drug-stimulations observed for pHRl and pHR6, their occurrence is at least qualitatively consistent with the evidence that transcription of rplKALJ (in common with other ribosomal protein operons) is stimulated in E. coli by low concentrations of rifampicin, although much less strongly than for rpoBC (8).

Turning to the observation that P \text{L11} and P \text{L10} together give scarcely more expression of lacZ than P \text{L10} alone, the most interesting interpretation would be that when P \text{L11} is active, the downstream P \text{L10} promoter is effectively "switched off" (a possibility first mooted as a speculation (20), and recently supported by S1-mapping of E. coli mRNA (30; also C. Squires, pers. commun.)). The mechanism of this effect is intriguing. It is most unlikely that P \text{L11} initiates so frequently as to produce close-packing of RNA polymerase molecules in the P \text{L10} region, and consequent steric exclusion of initia-
Table 2. cutdown by terminators fused to different promoters

<table>
<thead>
<tr>
<th>Promoter:</th>
<th>( P_{gal} )</th>
<th>( P_{ara} )</th>
<th>( P_{L11}/P_{L10} )</th>
<th>Natural</th>
</tr>
</thead>
<tbody>
<tr>
<td>rpo-tl</td>
<td>94 (pHR11)</td>
<td>98 (pHR4)</td>
<td>88 (pHR7)</td>
<td>82 (±3)</td>
</tr>
<tr>
<td>T7-tecl</td>
<td>77 (pIG125)</td>
<td>-</td>
<td>-</td>
<td>80 (±10)</td>
</tr>
</tbody>
</table>

(a) Ref. 25; also 26 (for \( P_{L10} \) alone).
(b) Ref. 27.

The plasmids carrying the relevant fusions are shown in brackets.

Translational properties of mRNA encoded by the fusions, as predicted by the known nucleotide sequences of the linkers, the rpl region (20), and trpB (19); and see ref. 5 regarding \( P_{gal} \) and T7 tecl:

- **Upstream** of the terminators: ribosomes should halt at the normal (rplL) stop codon, 69 nucleotides before rpo-tl, in pHR11, but should read 25 nucleotides further (into a potential stem-loop preceding the terminator stem-loop structure) in pHR4 and pHR7. Translation should halt some 200 nucleotides before the normal point in pIG125 (but in another \( P_{gal}/T7 \) tecl fusion, constructed by I.C., ribosomes should reach the normal \( gene \ 1.3 \) stop codon close to tecl; the cutdown observed is unchanged, 82%).

- **Downstream**: the \( P_{gal} \) fusions were made in a vector which prevents significant translational variations downstream (5). In pHR4 and pHR7 there should be no translation of trpB downstream of the insert.

Finally, Table 2 summarises the degrees of cutdown of gene expression produced in vivo by two different DNA fragments carrying transcriptional terminators, when inserted between various promoters and the genes monitored. The data are derived from Table 1 and ref. 5. The T7 "early" terminator (tecl) produces similar cutdown when fused to \( P_{gal} \) in plasmids, or to the phage's own promoters. However, the rpo-tl terminator fragment produces stronger cutdown when fused to \( P_{gal} \) (20-fold) or \( P_{ara} \) (50-fold) than to \( P_{L11} \) and \( P_{L10} \) in our plasmid pHR7 (8-fold), or in the normal chromosome (25), or \( P_{L10} \) alone in another plasmid (26). In the latter two cases, mRNA assays suggested 5- to 6-fold termination at rpo-tl. There is no correlation between the observed variations in cutdown and the translational properties of the fusions, upstream or downstream of the insert (see Legend to Table 2).

Despite the different results reported for \( P_{ara}/rpo-tl \) fusions by Barry et al (14), it is interesting to speculate that an anti-termination system might be brought into play by DNA sequences at or near ribosomal protein promoters.
such as $P_{L11}$ (and $P_{L10}$). Such a system, if accompanied by attenuators downstream of the promoters, might play a role in the regulation of ribosomal gene expression (as suggested by Post et al., 20; and for rpoD (sigma) gene regulation by Nakamura et al., 31). We have already discussed the probable existence of an attenuator just downstream of $P_{L11}$. Interestingly, recent evidence (28, 7) suggests that stringent control of transcription from ribosomal operon promoters might involve effects of ppGpp on "pausing" or attenuation.

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REFERENCES
1. Andrew J. Newman is now at the Department of Chemistry, S-010, University of California, San Diego, La Jolla, California 92093, USA.
2. Anne Wallis is now at the Department of Biochemistry, University of British Columbia, Vancouver, BC, Canada.