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Evidence that rifampicin can stimulate readthrough of transcriptional terminators in *Escherichia coli*, including the attenuator of the *rpoBC* operon

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ABSTRACT

The genes encoding the β and β' subunits of RNA polymerase in E.coli, rpoB and rpoC, lie downstream of at least two ribosomal protein genes, rplJ (encoding L10) and rplL (L7/12), in a common operon. All four genes are served by promoter P_{L10}, and an attenuator (partial terminator) of transcription, t1, lies between rplJL and rpoBC. Treatment of E.coli with rifampicin, under conditions producing partial inhibition of general RNA synthesis, can stimulate transcription of rpoBC. We have investigated the locus of this effect by fusing P_{L10} and t1 separately to galK, in suitable plasmids. Our studies of these fusions, and similar fusions involving transcriptional terminators derived from coliphage T7, indicate that low concentrations of rifampicin cause increased readthrough of several different transcriptional terminators in E.coli in vivo, including rpo t1. We discuss whether or not this unspecific mechanism is solely responsible for the observed stimulatory effects of the drug on rpoBC transcription.

INTRODUCTION

The β and β' subunits of RNA polymerase in E.coli are encoded by the genes rpoB and rpoC, which share a complex operon with four ribosomal protein genes rplK (L11), rplA (L1), rplJ (L10), and rplL (L7/12). The structure of this operon, shown in Fig. 1, is such that the downstream polymerase genes are obligatorily co-transcribed with rplJL (and perhaps rplKA): see reviews by Yura and Ishihama (1) and Matzura (2), also refs. 3-7. This arrangement presumably helps the cell to coordinate production of transcriptional and translational elements of the machinery for gene expression. There is evidence, however, that certain growth constraints can partially uncouple the expression of the rpo and rpl genes in the above operon (reviewed in 1, 2, 8, 9). One such constraint is produced by the drug rifampicin, which binds to RNA polymerase and blocks initiation (but not elongation) of the RNA product (10, 11). Partial inhibition of general RNA synthesis by rifampicin treatment of E.coli leads to a transient

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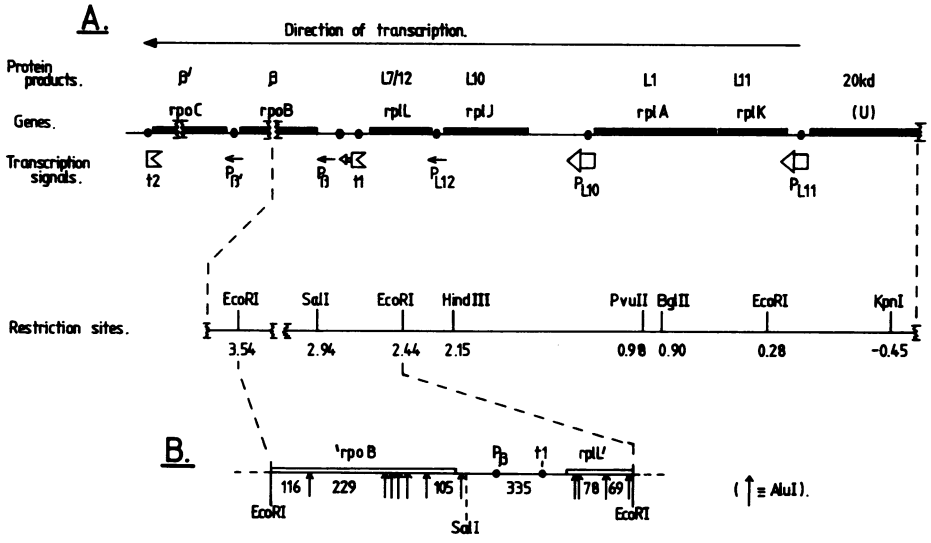

Fig. 1 Maps (to scale) showing the genes, transcriptional signals, protein products, and targets for relevant restriction enzymes, in segments of λrif^{d}18 DNA carrying (A) the rplKAJL rpoBC operon and its environs and (B), in more detail, the EcoRI fragment spanning the rplL-rpoB junction; derived principally from Post et al (39): see also refs. 3-7. No BamHI targets occur. In (A), U represents the gene coding for a 20 kd polypeptide of unknown function. Other gene symbols are explained in the Introduction. The coordinates shown on the restriction map in A are in kilo basepairs, on the scale established by Post et al (39): fragment sizes in B are given as basepairs. • : map position of transcription signal (tentative for P_{L12} and $P_{\beta'}$); ⇦ : strong promoter; ← : weak promoter; ⇦ : partial terminator; ⇦ : strong terminator.

absolute increase in the rate of synthesis of β and β' (and other polymerase subunits) without a parallel effect on the ribosomal proteins L7/12 encoded upstream in the ββ' operon (12-16). In fact the transcription of the rpoBC genes is strongly stimulated, while that of the rplKAJL region and other ribosomal RNA and protein genes displays weak stimulation (17, 18, 8). It is possible that post-transcriptional events play a minor role in the effects of rifampicin on β and β' synthesis (8, 18, 19), but the main effects in vivo are clearly transcriptional (17).

In an attempt to define the regulatory site(s) mediating transcriptional stimulation of rpoBC, we have exploited recombination in vitro to fuse the shared promoter of the rplJL and rpo genes (P_{L10}), and the partial terminator (t1) which lies between rplJL and rpoBC, separately to galK in plasmids derived from pKO1 (20). We have also fused galK to some transcrip-

tional terminators from coliphage T7. We have then examined the effects of low levels of rifampicin on expression of the indicator gene product by strains harbouring these fusion plasmids, to test whether the drug leads to stimulation of the promoter P_{L10} and/or to decreased efficiency of termination at t1, and to examine the specificity of any such effect. Our results indicate an unexpected, general effect of this drug on termination of transcription.

MATERIALS AND METHODS

Strains. The following strains were used: E.coli K12 ED8654 (hsdR hsdM$^+$ supE supF) from Dr. N. Murray; E.coli K12 N100: galK recA56 pro his (20); and E.coli B-HB101 (leu pro lac gal thi strA recA hsdR hsdM)(21). The plasmids used included pNA26 and pNA38, carrying respectively the 1.1 kb ('rpoB-rplL') EcoRI fragment of λrif^{d}18 DNA (Fig. 1) in pSF2124 (21) and the 1.87 kb ('rplJ-rplK') Hind III-EcoRI fragment of λrif^{d}18 DNA in pBR322 (22). pNA26 and pNA38 were constructed in our laboratory by Dr. A. Nicolaidis. The plasmid vectors employed for the analysis of promoters and terminators were pKO1 and pKG1800 (20). λrif^{d}18 is a defective transducing phage carrying the rrnB-rpoC region of the E.coliK12 chromosome, including a dominant Rif-R allele of rpoB (24, 25). λ590 (λb538 imm434 shn6^o, from Dr. Noreen Murray) is an insertion vector for HindIII-generated DNA fragments (26). Phage T7, and its HpaI-G (446 bp) fragment cloned with BamHI molecular linkers into pBR322 to generate plasmid pAR48 (27), were from Dr. F.W. Studier.

Phage, plasmid and DNA manipulations were mainly carried out as described previously (6, 28). Small scale plasmid preparations were based on ref. 29; large scale preparations, on ref. 30. Chloramphenicol was usually omitted. pNA26 and pNA38 were propagated in E.coli HB101; pKO1, pKG1800, and their derivatives in N100.

Construction of pHR1800 and pHR9. The multicopy plasmid pKO1 (20) carries an intact E.coli galK gene downstream of the following relevant features (cf. Fig. 2): unique EcoRI, HindIII, amd SmaI sites in succession, followed by DNA encoding translational stop codons in all three phases. pKO4 (McKenney, pers. comm.) is identical but for the presence of a BamHI site inserted (on a "linker") at the SmaI target of pKO1. pKG1800 (20) is closely related to pKO1, but carries the gal promoter of E.coli on a 1.09 kb EcoRI-HindIII replacement fragment; it therefore expresses galK vigorously in E.coli N100, whereas pKO1 carries no significant promoter. However,

pKG1800 differs from pKO1 in one other respect, disadvantageous for our purposes; a stop codon in one phase of translation is not reached before the ribosome-binding site of galK.

We constructed pHR1800, a version of pKG1800 having stop codons in all three phases upstream of galK,by transferring the gal promoter (EcoRI-HindIII) fragment from pKG1800 into pKO1, as a replacement. pKG1800 (cut with EcoRI, HindIII, and PstI endonucleases) was ligated with pKO1 (digested with the former two enzymes). Amp-R, Gal^+ transformants of E.coli N100 were screened by HaeII-, EcoRI-, and HindIII-digestion of small-scale plasmid preparations to identify the required pHR1800 (Fig. 2). It has the "destroyed HaeII-site" characteristic of pKO1 (20), and should therefore encode stop codons in all three phases, well upstream of galK . As expected, N100 (pKG1800) and N100 (pHR1800) produce indistinguishable amounts of galactokinase (data not shown).

pHR9 is a derivative of pKO4, carrying the EcoRI-(gal promoter)-HindIII fragment from pKG1800 as a replacement; thus it is identical to pHR1800, except for the presence of the BamHI linker characteristic of pKO4 (see above). We constructed pHR9 as for pHR1800, but using pKO4 in place of pKO1, and pHR1800 instead of pKG1800.

Construction of λAJN81. A 335 bp AluI fragment carrying the t1 attenuator from the rpoBC operon was adapted to facilitate construction of fusions to galK, by addition of HindIII molecular linkers (dCCAAGCTTGG) to its termini. The donor DNA was AluI-digested pHR2; pHR2 is a pBR322 -derivative carrying the 1.1 Kb 'rplL-rpoB' EcoRI fragment (Fig. 1), transferred from pNA26. 2 µg of linker were phosphorylated, and ligated with 4 µg of donor, as described by Maniatis et al (31). The mixture was then incubated 10 min x 70°C, and digested (1 h x 37°C) with 40 units of HindIII. Linker fragments were removed by gel filtration (Sephadex-G75), and eluted DNA >50 bp in length was cloned into the HindIII-site of λ590 (26). After transfection into ED8654, clear plaques were picked and screened by probing with ^{32}P-labelled 1.1 Kb EcoRI fragment (from pNA26). The relevant product was a recombinant, λAJN81, carrying a 345 bp HindIII fragment. That it includes the rpot1 (AluI) fragment was confirmed by the fact that it introduces an extra SalI target into λ590 DNA. This AluI fragment contains the only SalI site present in the donor plasmid pHR2 (see Fig. 1; and ref. 32 for pBR322).

Galactokinase assays. These were based on refs. 33 and 20. The plasmid-bearing strains were grown at 37°C in Spizizen minimal medium containing 0.2% fructose as carbon source (to avoid catabolite repression), 0.1% casa-

mino acids, and ampicillin at 50 µg/ml. The doubling times for all strains studied were between 60 and 70 min. Assays were carried out at an initial A^{650} between 0.2 and 0.25. 1 ml samples were treated with 40 µl of lysis buffer (100 mM EDTA, 100 mM DTT (dithiothreitol), 50 mM Tris HCl, pH8.0) plus two drops of toluene, and vortex-mixed for 1 min. Toluene was evaporated off using an air-line (20°C); lysates could then be stored on ice for at least 60 min prior to assay. 20 µl aliquots were mixed with 80 µl of reaction mixture comprising 20 µl of 5 mM DTT/16 mM NaF; 50 µl of 8 mM $MgCl_2$/200 mM Tris.HCl (pH7.9)/3.2 mM ATP; and 10 µl of 10 mM D-galactose containing D-(1-^{14}C) galactose (Amersham) at a final specific activity of 4.5×10^6 dpm per µmole. (The diluted ^{14}C-galactose is filtered twice through DE81 before use). After incubation at 32°C for 10-30 min, 50 µl reaction samples were removed to 2.5 cm discs of DE81 paper, and washed (34) in d.H_2O (3 x 20 min, 20°C). Blank filters were washed simultaneously, and two 25 µl reaction samples taken at random were transferred to DE81 discs and remained unwashed (to determine total counts). Filters were dried (95°C, 15 min) and counted by scintillation in 0.4% butyl-PBD (CIBA)/toluene. Galactokinase units (nanomoles galactose phosphorylated per min per A^{650}) = (cpm - blank) x 2500, divided by (average of unwashed filters x time of incubation x A^{650}). The assay is linear with enzyme input up to 25% conversion of the substrate. Note that addition of galactose or fucose to these strains has little or no effect on galactokinase synthesis: presumably the gal operon is effectively derepressed by its presence in high copy-number (galR being in single copy).

Sources of materials. Restriction endonucleases were purchased from Boehringer Corporation Ltd., Bethesda Research Labs., or New England Biolabs, or prepared in this Department. We purified AluI from Arthrobacter luteus, ATCC 21606, as described by Roberts et al (1976), including the final DE-cellulose step which significantly reduces exonuclease contamination. The enzymes were used under the conditions prescribed by the commercial suppliers. T4 DNA ligase was from New England Biolabs, T4 polynucleotide kinase from Boehringer, and calf intestinal phosphatase from PL Biochemicals. The molecular recombination linker (dCCAAGCTTGG) was from Collaborative Research Inc. D-(1-^{14}C) galactose was from Amersham, and DE81 paper from Whatman. Pancreatic DNase and RNase were from Sigma. Other special chemicals were variously from BDH, Serva, or Sigma.

RESULTS.

Construction of fusions to galK. The ingeniously designed multicopy plasmid

pKO1 (20) carries the E.coli galK gene downstream of the following relevant features: unique EcoRI, HindIII, and SmaI sites in succession, followed by DNA encoding stop codons in all three possible phases of translation. Thus, irrespective of the DNA inserted, ribosomes should halt translation of derived mRNA at least 74 nucleotides before the start codon for galK. For this and other reasons (discussed in 20) polarity effects arising from fusions should be minimal. pKO4 is identical to pKO1, but with a BamHI molecular linker inserted at the SmaI site; pKO6 has an EcoRI linker at this site, and the EcoRI site of pKO1 is replaced by a BamHI site. (pKO6 was constructed by G. Christie and T. Platt). In all three plasmids the easily assayable galactokinase product is very poorly expressed, since no strong promoter for galK is present. pHR1800 and pHR9 (Materials and Methods, and Fig. 2) are derivatives of pKO1 and pKO4, respectively, in which galK is served by the homologous promoter P_{gal}.

The 335 bp AluI fragment cloned (with HindIII linkers) in λ AJN81 (see Materials and Methods) carries the "carboxy-terminal" 23 nucleotides of rplL, and most of the rplL-rpoB intercistronic region, including terminator t1; it ends 12 nucleotides short of rpoB (Fig. 1). To fuse t1 to galK we ligated HindIII-digested λ AJN81 DNA with pHR1800 digested by HindIII and calf intestinal phosphatase. The products were used to transform E.coli N100 (galK recA) to Amp-R. Restriction analyses with HindIII, EcoRI, and SalI (not shown) established that one plasmid derived from these transformants, pHR11, has the rpot1 fragment inserted in the normal transcriptional orientation between Pgal and galK of pHR1800 (Fig. 2); pHR12 has this insert in the opposite orientation.

To fuse galK to the P_{L10} promoter of the rpoBC operon we replaced the short BamHI-HindIII segment of pKO6 with the 1.25 Kb BglII - HindIII ('rplA-P_{L10}-rplJ')-fragment of λrif^d18 DNA (Fig. 1). The immediate donor was plasmid pHR1, a pMC81-derivative carrying a more extensive region of λrif^d18, whose construction is described in the accompanying paper (35); we digested it with BglII and HindIII endonucleases, plus EcoRI to sever unwanted fragments. After ligation Amp-R, Gal^+ transformants of E.coli N100 were selected (N100/pKO6 is Gal^-). One such colony contained plasmid pHR8, shown by restriction analyses and "Southern" hybridisations to have the structure given in Fig. 2. Accordingly galK expression should be dependent on transcription initiated at P_{L10}.

We have also cloned fragments of coliphage T7 DNA carrying terminators of transcription into the HindIII site of pHR1800, or the BamHI site of

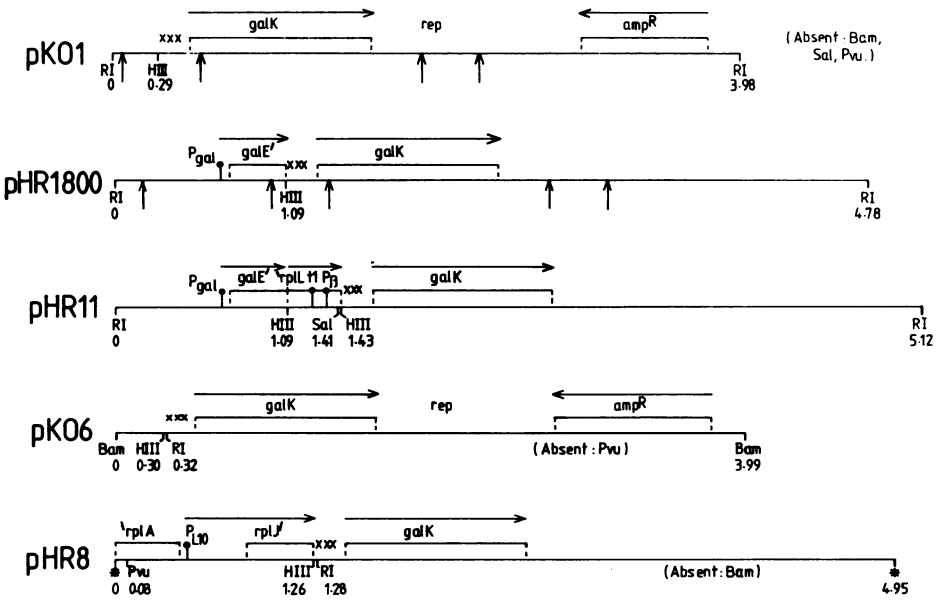

Fig. 2. Maps (to scale) of the vectors pKO1 (20), pHR1800 (ibid., and see Materials and Methods), and pKO6 (McKenney, pers.commun.), and of our derivatives containing inserted DNA from the rpoBC region of λrif^{d}18. Only the restriction targets relevant for construction or characterisation of our fusion plasmids are shown on each map (with their coordinates in kilo-base pairs). Relevant genes, transcriptional signals, and normal transcriptional orientations (horizontal arrows) are also shown. All plasmids are drawn as if linearised at the single EcoRI site, or its BamHI equivalent in pKO6 and pHR8. xxx: stop codons in all 3 phases of translation; Bam: BamHI-restriction target; HIII: HindIII-; Pvu: PvuII-; RI: EcoRI-; Sal: SalI-; *: BamHI/BglII hybrid site; ↑: HaeII-target. We have directly verified all of the restriction maps shown.
Note: The structure of pHR8 was confirmed by "Southern" hybridisation applied to a HindIII + PvuII-digest, using ^{32}P-pBR322 DNA and ^{32}P-pNA38 DNA as probes. The latter hybridised strongly to both the 1.18 Kb (rpl'AJ') band and the 3.77 Kb band, whereas pBR322-DNA hybridised significantly only to the 3.77 Kb band. (pKO1 and pKO6 are derived from pBR322).
pHR12 is identical to pHR11, but has the opposite orientation of the "t1" insert (Sal site at 1.11 Kb). pKO4 is identical to pKO1, but for the insertion of a Bam site at 0.32 Kb. pHR9 is identical to pHR1800, but with a Bam site at 1.12 Kb.

pHR9, between Pgal and galK. Details of the construction of these fusions will be reported elsewhere. The main "early" terminator of T7 (T7 tec1) is carried on a 446 bp HpaI-fragment, with BamHI linkers, in the plasmid pAR48 (27, 36). We inserted this fragment into the BamHI site of pHR9, to generate pIG125 (with the terminator oriented "normally" with respect to galK transcription) and pIG126 (with the opposite orientation of the insert).

A newly discovered terminator of transcription (I.G., unpublished) is present in a 179 bp AluI-fragment of T7 DNA. Southern-blot hybridisation, DNA-sequencing, restriction analyses, and comparison with unpublished T7 DNA sequence data (generously provided by Drs. J. Dunn and F.W. Studier) show that this fragment contains nucleotides 29429 to 29607 of the T7 genome. It includes the HpaI site at 29587 (74.09%), and a putative terminator signal of conventional structure (cf. 37). This terminator, which we name T7 tec3 (terminator for E.coli polymerase), is so oriented in T7 DNA that it would stop transcription only in a leftward direction. There is no evidence that it has any physiological role. We inserted it (with HindIII linkers) into pHR1800 to generate pIG116 (with T7 tec3 in its functioning orientation with respect to galK transcription) and pIG115 (inverse orientation). Expression of galactokinase by the fusion plasmids. Table 1 summarises our measurements of galactokinase synthesis by exponential cultures of E.coli N100 growing in fructose-minimal medium (20) and harbouring various plasmids. Assuming that the observed results reflect transcriptional effects, it is evident that the fragment of rpoDNA carrying t1 produces efficient (20-fold) termination in pHR11. The newly discovered T7 terminator, tec3, is efficient in pIG116. It appears to be inactive when inverted (pIG115); inspection of the nucleotide sequences shows that ribosomes (from galE' (20)) would be expected to read through the mRNA corresponding to the entire insert in pIG115, which might prevent any potential transcriptional terminator from functioning. The classic T7 early terminator, "tec1", appears to cause 77% termination in pIG125, in line with evidence obtained for T7 itself in vivo (38). Unexpectedly, inversion of the tec1 fragment produces even more efficient termination (pIG126); similarly, the inverted rpot1 fragment causes termination (pHR12). The RNA stop-site in both cases is unknown. The sequence of the rpot1 terminator region (4, 39) is such that it might well cause termination when inverted, assuming the validity of the consensus sequence ascribed to "rho-independent" terminators (37). However, the "stem" component of T7 tec1 includes a central G.U basepair (36); since A cannot pair with C, it is unlikely that the tec1 sequence per se could provide a functional stem component of a termination signal when inverted. Inspection of the overall sequence suggests that the terminator in this case may be generated by the fusion made, rather than being internal to the "inverted" T7 DNA fragment.

The result for pHR8 suggests that P_{L10} may be about half as efficient as P_{gal}. However, this could be an under-estimate. pHR8 is an unstable

Table 1.

Plasmid harboured by E.coli N100.	Known promoters and terminators affecting galK[c]	Galactokinase activity (U/A^{650})[a]	Extent of cutdown (%)[d]
pHR1800[b]	P_{gal}	570 (±21)	-
pHR 11	P_{gal} rpotl $P\beta$ →	33 (±3)	94
pHR12	P_{gal} ← P_{β} rpotl	155 (±10)	73
pIG116	P_{gal} T7 tec3 - - - - →	30 (±4)	95
pIG115	P_{gal} T7 tec3 ← - - - -	544 (±16)	4.6
pHR9[b]	P_{gal}	745 (±40)	-
pIG125	P_{gal} T7 tecl →	171 (±3)	77
pIG126	P_{gal} T7 tecl ←	15.3(±0.3)	98
pHR8	P_{L10}	374 (±19)	-

[a] Activity determined using exponential cultures in fructose minimal medium with ampicillin, grown without dilution for at least 18 hr prior to assaying at A^{650} between 0.2 and 0.45. The standard error of the mean is shown in brackets: except for pHR8, at least 16 separate assays and 4 independent cultures were used.

[b] pHR1800 is the proper control for pHR11,-12, pIG116,-115; pHR9 is the proper control for pIG125 and pIG126.

[c] The superscript arrows show (where known) whether the terminator is orientated with respect to transcription as in its normal environment (→) or in the opposite sense (←). tec3 may not actually function in T7.

[d] Reduction of enzyme activity ascribable to the terminator insert.

plasmid, readily lost during growth in liquid minimal medium. Early experiments gave highly variable results for galactokinase production. In the experiment included in Table 1, all cells contained the plasmid (as judged by viable counts on broth plates ± ampicillin). However, we cannot exclude the possibility of an abnormally low copy number per cell. DNA yields from N100 (pHR8) have been consistently low. For all the other galK plasmids studied here (except pHR9) there have been no indications of copy number variations or instability. Instability of pHR8 might arise from production of galE-rplA or rplJ-plasmid hybrid proteins. These might mimic L1 or L10 sufficiently well to disturb ribosome assembly, and/or to repress translation of L1, L11, L10, and L7/12. Alternatively the latter auto-repression (reviewed in 9) might be seriously disturbed because the "galK"-mRNA pro-

duced by pHR8 carries an extra dose of the target site for repression, by L10, of rplJL-translation.

The difference in galactokinase production between pHR9 and pHR1800 seems reproducible, and may be related to an unusually high copy number of pHR9 (judged by plasmid yields). If so, it is not clear why mere insertion of a BamHI linker between P_{gal} and the 3-phase stop codons should produce such effects.

Effects of rifampicin on the galK fusions. Low levels of rifampicin partially inhibit the initiation of transcription in general, but specifically stimulate rpoBC-mRNA synthesis in vivo (17). Does galK expression show an analogous response when linked to rpo DNA?

The effects of adding low concentrations of rifampicin on the accumulation of galactokinase were examined for E.coli N100 growing exponentially in fructose minimal medium and harbouring various galK plasmids. The standard level of rifampicin used (10 µg per ml) has no detectable effect on growth of any of these strains over the course of a 50 min experiment. At 20 µg per ml, growth is inhibited significantly (A^{650} reduced by 10% relative to the control, after 50 min). However, the effects on galactokinase synthesis were the same at 10 and 20 µg rifampicin per ml, for all strains tested (pHR1800, pHR8, pIG116, pIG125). Addition of dimethylformamide alone (the solvent for rifampicin) had no effect (tested with pIG126). The results obtained with 10 µg drug per ml are summarised in Fig. 3.

Addition of rifampicin has no detectable effect on galactokinase accumulation in strains carrying plasmids without a functioning transcriptional terminator upstream of galK: namely the pHR1800, pHR8, and pIG115 strains (Fig. 3A,D, and B: also pHR9, not shown). Note the implication that rifampicin does not stimulate P_{L10} (in pHR8). However, with all five plasmids carrying a known or putative transcriptional terminator (Fig. 3C, and E-H) there is a striking stimulation of enzyme synthesis by the drug. The strength of this effect is roughly correlated with the efficiency of the terminator, at least for T7tec1 (Fig. 3F) as compared with T7tec3 and the putative terminator(s) arising from the inverted T7tec1 fragment (Fig. 3H, G). The simplest conclusion is that rifampicin, when present at low concentrations in vivo, increases readthrough of transcriptional terminators in general. Note, however that the effect of the drug may be established more rapidly for rpot1 than for the other terminators: compare Fig. 3E (rpot1) with Figs. 3F, G, and H (T7tec1 inverted, T7tec1, T7tec3). Note also that rpot1 does not require P_{L10} in order to show stimulation by rifampicin.

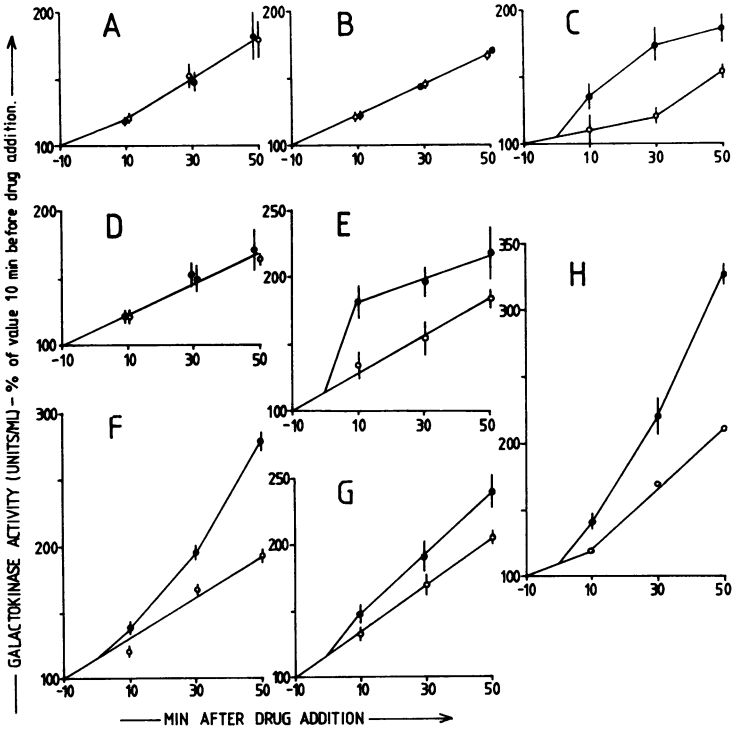

Fig. 3. Effect of rifampicin (10 µg per ml) addition upon galactokinase accumulation in E.coli N100 harbouring pHR1800 and various derivatives. Activity (units/ml) is plotted as a percentage of the pre-drug level, versus time after drug addition. o:no drug; •:with drug. Vertical bars indicate standard errors of means derived from 3 to 6 separate cultures, each assayed in duplicate (except for pIG126 and -125: two separate cultures each). The plasmids, and the known or putative termination sites affecting galK expression in each case are as follows: A, pHR1800 (none); B, pIG115 (none: T7tec3 fragment inverted); C, pHR12 (rpot1 fragment inverted); D, pHR8 (none; P_{L10} responsible for initiation); E, pHR11 (rpot1); F, pIG126 (T7tec1 fragment inverted); G, pIG125 (T7tec1); H, pIG116 (T7tec3). In all cases except D, P_{gal} is responsible for initiation of transcription.

DISCUSSION

Throughout this discussion we shall assume that the observed effects of inserted DNA on the expression of galactokinase by the plasmid-encoded genes arise at the level of transcription. Although mRNA analyses will be necessary to prove this, it is a plausible assumption. In the galK plasmids, ribosomes "reading out" of the various transcribed inserts should be unable to affect transcription or translation differentially (20). It is true that an individual fusion could conceivably affect the efficiency of trans-

lation of galK mRNA through abnormal RNA secondary structure, or by introducing new RNA-processing sites such as the RNAaseIII-target encoded in the rpotl fragment (4). This could lead to errors in the estimation of individual terminator strengths, but it seems implausible that it could mimic stimulation by rifampicin of transcriptional readthrough for several different DNA fragments. Moreover the rifampicin effects have also been observed in our studies of fusions to lacZ, described in the accompanying paper (35). Finally, we have reason to believe (see Results) that plasmid copy-number variations have no important bearing on the points discussed below.

It should be emphasised that the source of rpoDNA used here (and in most published work) is λrif^{d}_{18}. The nature of the rif^{d}_{18} mutation(s) remains unclear (6); rpotl could conceivably be affected. However, the observed rifampicin effects are not unique to rpotl, and are therefore not peculiar to rif^{d}_{18} DNA.

Table 1 shows that the fragment of rif^{d}_{18} DNA carrying the "attenuator" tl produces remarkably efficient termination of transcription (20-fold) in pHR11. Previous reports indicated a 5 to 6-fold efficiency in the wild-type chromosome (40) and for rif^{d}_{18} DNA in multi-copy plasmids (4, 41). The pKO plasmids prohibit downstream polar translation effects of fusions on galK expression (20). Moreover, in mRNA derived from pHR11 ribosomes from galE (20) should enter the tl region in normal rplL phase (39) and stop at the usual position (although not necessarily in the normal quantities). Thus operation of tl is unlikely to be disturbed by the translation of the upstream mRNA regions. One possible explanation for the high efficiency of rpotl in pHR11 would implicate the foreign promoter, as discussed in the accompanying paper (35).

Turning to the striking effects of rifampicin on gene expression by the fusion plasmids, the simplest hypothesis which can be made to explain our results is that this drug, when present at low concentration in cultures of normal (Rif-S) E.coli, stimulates readthrough of transcriptional terminators in general. What could be the mechanism of such a general effect? There is evidence that rifampicin can bind stoichiometrically to RNA polymerase which is actively transcribing, without inhibiting elongation (11). Thus it seems possible that the drug could bind to enzyme molecules after they have initiated transcription in our in vivo conditions (either during elongation, or when the polymerase has paused at a terminator), and could subsequently interfere directly in some general step of termination (reviewed in 42).

This is all the more plausible if one recalls that *rif*^{r} mutations can affect termination positively or negatively (eg. 52). However, indirect effects (*e.g.* on the synthesis of a general termination factor such as *nusA*-product; see 42) cannot be excluded; note the slow kinetics observed for several of the fusions (Fig. 3). Tittawella (43) presented evidence favouring an indirect mechanism for rifampicin-induction of ββ' synthesis. Both T7tec1 (44) and *rpot*1 (8) appear to be unaffected by *rho*. We have not yet tested a known rho-dependent terminator for rifampicin effects.

The results for pHR8 indicate that rifampicin in low concentration *in vivo* does not stimulate initiation at the strong *rpoBC* promoter, P_{L10}. Our studies of *lacZ* fusions (35) have shown that the weak promoter P_β is also not detectably stimulated. Can the effects of low levels of the drug on normal, haploid *E.coli* cells be explained solely by unspecific readthrough of *rpot*1 and other terminators? If so, many genes should be affected, and evidence of this has been published recently (8; see also 45). The detailed effects on the wild-type *rpoBC* operon were studied by Blumenthal and Dennis (17). *rpoBC*-transcription was stimulated by 150%; but *rplKAJL*-transcription was also stimulated, although only by 30-40%, as for several other ribosomal operons. The increased readthrough of *rpot*1 which we have observed could explain the strong relative stimulation for *rpoBC*. In principle, the weak stimulation of *rplKAJL* might stem from some partial terminator lying, for example, between P_{L11} and the proximal ribosomal genes (see refs. 35 and 39). Thus it is possible to explain the effects of rifampicin on *rpoBC* transcription, at least qualitatively, without invoking any specific autogenous regulatory mechanism such as we originally proposed (12, 13). The inability of streptolydigin to stimulate ββ' synthesis (13) is easily understood on this model; an inhibitor of elongation (46, 47) would hardly be expected to increase readthrough of terminators. Finally, the transient stimulation of α- and σ-synthesis by rifampicin (14, 16) could be due to a directly similar mechanism, or to indirect effects of *rpoBC*-stimulation.

Can the evidence for a specific autogenous regulation of *rpoBC* transcription (reviewed in 48, 1, 2, 8, 9) now be wholly discounted? Evidently, temperature-sensitive RNA polymerase core mutations could produce their effects on *rpoBC* transcription through a general alteration of termination efficiency; for example, recent detailed studies of an *rpoC*^{ts} mutant at semi-permissive temperature (49) indicate transcriptional alterations closely resembling those produced by rifampicin. However, similar altera-

tions have been observed upon shifting a temperature-sensitive sigma mutant to 42°C (50); a direct effect on transcriptional termination would not be predicted in this case, although it cannot be excluded. More strikingly, the uncoupling effect of partial amino acid starvation in relA$^+$ strains, where rplKAJL transcription is reduced but rpoBC transcription is unaffected (51), would be difficult to explain by a direct, unspecific alteration of terminator efficiency. If anything, the accumulation of ppGpp associated with starvation in these strains might be expected to increase pausing at terminators (23) thus perhaps decreasing rpoBC transcription relative to rplKAJL. Since this kind of constraint is likely to be encountered by E.coli in its wild state, the observed response of rpoBC might well be physiologically significant, and it remains suggestive of a possible specific autogenous regulation of transcription of these genes. Other evidence of such a "compensatory" regulation, derived especially from studies of poorly suppressed rpoB amber mutations, is summarised in the above reviews.

Finally, we see two reasons not to dismiss the possibility of a special effect of rifampicin on the rpotl attenuator. In the first place the kinetics of the response of rpotl in our galK fusion seem to differ from those of other terminators. The major stimulation occurs sooner, and there is a suggestion of transience reminiscent of the effect of the drug on ββ' synthesis directed by the chromosome. Secondly, if we are correct in assuming that the general readthrough effect of rifampicin requires that the drug should bind to sensitive enzyme molecules only after they have initiated RNA synthesis, we might not expect to find any such effect at high drug concentrations, when sensitive molecules should be unable to initiate. Indeed we have recently found that stimulation of galK expression is confined to the first 5 min after addition of 100 μg rifampicin/ml to N100 (pHR11) and -(pIG116); whereas stimulation of ββ' production is strongest after 10 to 15 min when various rif^S/rif^R heterodiploid strains are similarly treated (12, 13, 16). Thus it is possible that the drug has a second, specific effect on the rpoBC operon. Studies of our fusion plasmids in a heterodiploid strain should provide a test of this hypothesis.

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