Expression of the *E.coli* hemolysin secretion gene *hlyB* involves transcript anti-termination within the *hly* operon

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ABSTRACT

The genes *hly A* and *hly B* dictating synthesis and secretion of hemolytic toxin by *Escherichia coli* are transcribed as part of an operon *hly C, hly A, hly B* but are separated by an inverted repeat and poly-T sequence characteristic of a rho-independent terminator. The *hly A-hly B* intergenic sequence caused a reduction of *in vivo* transcriptional read-through from the *gal* promoter into *gal K* when inserted in the pKG1900 terminator-probe vector and also *in vitro* 3' to the bacteriophage T7 σ 10 promoter. Hybridisation of mRNA generated by the *hly* determinant of recombinant DNA pBR202-312 to an antisense RNA probe spanning the *hly* intergenic sequence revealed specific termination of 80% of *in vivo hly* transcripts within the poly-T sequence, immediately preceeding the *hly B* translation start. Extension of the *hly* determinant with 3.5kbp of *hly* promoter-proximal DNA sequence raised intracellular and cell-free hemolytic activity 3-fold and 20-fold respectively and increased markedly the secretion of the 107Kd hemolysin protein (HlyA). Parallel hybridisation of *in vivo* mRNA to *hly A* and *hly B* antisense probes revealed that levels of *hly A* and *hly B* mRNA were increased by approximately 3-fold and 90-fold. The dramatically enhanced level of *hly B* mRNA resulted primarily from specific suppression of transcription termination at the intergenic rho-independent terminator from 80% to 1%, thus allowing virtually all *hly A* transcripts to elongate into *hly B*.

INTRODUCTION

The *Escherichia coli* hemolysin (*hly*) determinant encodes the synthesis of cytotoxic hemolysin and its secretion across both bacterial membranes without the aid of an N-terminal translocation signal or peptide cleavage (1,2). The genes for the synthesis (*hly A*) and cytoplasmic post-translational activation (*hly C*) of cytotoxic hemolysin have been shown to be transcribed in an operon together with one of the 2 contiguous specific secretion genes, *hly B* (3,4,5). This gene encodes HlyB, a putative ATP-binding membrane protein analogue of the mammalian P- glycoprotein (6,7), which together with a second membrane protein HlyD directs the complete export of hemolysin into the medium (8,9,10).

Transcription of the conserved hemolysin genes occurs in the order *hly C, hly A, hly B* from a promoter region preceeding *hly C* (3,11,12, fig. 1) but transcription of the synthesis and secretion genes is subject to pronounced operon polarity; the HlyB membrane protein is made at relatively low levels (11,13) and transcriptional activity measured by β -gal fusions is markedly lower within the *hly B* gene (4,5). In this report we demonstrate that strong operon polarity is caused by termination of *hly* transcription between the genes *hly A* and *hly B*, imposed specifically by an inverted repeat and poly-T sequence characteristic of a rho-independent terminator. We also show

that in the presence of DNA proximal to the *hly* operon promoter region, efficient expression of the *hly* B secretion gene is effected preferentially by the specific and strong suppression of transcription termination at this intergenic rho-independent terminator.

MATERIALS AND METHODS

Bacterial strains and plasmids.

E.coli strains HB101 and 5KC (*rec* A1 *hsd* R *hsd* D) were used in this study. Plasmid pBR202-312 was constructed from pBR322 and pANN202-312, which contains the *E.coli hly* determinant of the wild-type plasmid pHly152 (10,14); pEK50 contains the same *hly* determinant on a *Sal* I fragment of pHly152 but contains an additional ca.3460bp of *hly* promoter-proximal DNA, analogous to the plasmid described by Zabala *et al.* (15). Plasmids pKG19-t(+) and pKG19-t(-) were constructed by cloning the pBR202-312 477bp *Pvu* II-*Eco* RV restriction fragment, which contains the *hly* A-*hly* B intergenic region, into the *Sma* I site of terminator-probe vector pKG1900 (16) between the *E.coli gal* promoter and the *gal* K gene. The orientation of the inserted fragment is indicated by (+) or (-). Recombinant plasmids used for the preparation of anti-sense RNA probes were derived from the Genescribe vectors pT7-1 and pT7-2 (US Biochem. Corp.), which utilise the specific *in vitro* activation of the strong class III T7 bacteriophage ϕ 10 promoter by T7 RNA polymerase (a kind gift of Bill Studier, Brookhaven). The 477bp *Pvu* II-*Eco* RV fragment spanning the intergenic region was cloned in the correct and reverse orientations into the *Sma* I site of pT7-2 to give respectively pT7-t(+) and pT7-t(-). The *hly* A-internal 812bp *Hin* dIII -*Eco* RI fragment was cloned into the corresponding sites of pT7-1 to give pT7-*hly* A and the 799bp *Eco* RV-*Pst* I fragment was cloned into the polylinker *Sma* I-*Pst* I sites of pT7-2 to give pT7-*hly* B. The control measurement of *bla* transcript levels was made using antisense RNA generated from pT7-*bla*, pT7-2 carrying the *bla* -internal 296bp *Hinc*II-*Pst* I fragment.

Assay of hemolysin secretion.

(a) Hemolytic activity was measured during batch growth of *E.coli* 5KC transformants at 37°C, shaking in Brain Heart Infusion broth containing 50µg/ml ampicillin. Aliquots of culture were removed at intervals during exponential growth (A_{600} 0.2-0.85) and assayed for both intracellular and cell-free secreted activity in, respectively, a cleared cell extract of bacterial cells lysed by lysozyme treatment and supernatant from a cell-free centrifuged culture. Lysozyme treatment entailed resuspending pelleted washed cells from 1ml. of culture in 35µl. buffer (25mM HEPES-KOH, pH8.0, 5mM EGTA, 1mM DTT), freezing in liquid nitrogen, thawing at 10°C, then incubating with 3µl 2M KCl and 1µg/µl lysozyme at 0°C. Samples were refrozen and thawed, then diluted in HEPES buffer to the original culture volume. Samples were incubated for 15-30 min. at 40°C in a 2% suspension of erythrocytes in 0.85% NaCl, 20mM CaCl₂ after which hemoglobin released was measured at A_{543} . Activity was calculated as arbitrary hemolytic units (1 unit causing release of 50mg of hemoglobin in 1 hr. of the standard test).

(b) The extracellular presence of the previously well-characterised 110KDa hemolysin protein

in the above samples was assayed by concentrating 20ml. of cell-free medium by TCA (10% v/v on ice) precipitation of protein. Proteins were redissolved in 100 μ l SDS loading buffer, neutralized with saturated Tris base and heated in a boiling water bath for 10min. before separation on a 15% acrylamide gel and staining with coomassie blue.

Assay for galK activity.

Plasmid-bearing *E.coli* HB101 cells were grown in minimal M9CA media containing 0.2% D-fructose as carbon source at 32 $^{\circ}$ C to log. phase (A_{600} 0.6). Cells were lysed by 0.1M EDTA, 0.1M DTT, 50mM Tris-HCl, pH8.0 plus a drop of toluene at 40 $^{\circ}$ C for 20min. Galactokinase activity in the cell lysates was assayed (by phosphorylation of 14 C galactose, Amersham UK) as described (16).

Preparation of cellular RNA.

Cellular RNA was prepared from plasmid-bearing *E.coli* HB101 or 5KC as described previously (12), except that cells containing pKG1900 *gal* K recombinant plasmids were grown in minimal M9CA medium containing 0.2% D-fructose at 32 $^{\circ}$ C.

Preparation of anti-sense RNA probes.

Linearised recombinant DNAs were used as templates for the *in vitro* generation of full-length run-off transcripts. The plasmids pT7-t(+), pT7-t(-), pT7-*hly* B and pT7-*bla* were digested with *Eco* RI, the plasmid pT7-*hly* A was digested with *Hin* dIII. These linear DNAs were used as templates for RNA synthesis. High specific activity RNA probe synthesis was carried out using template DNA (0.2-1.0 μ g/ μ l) in 30 μ l 20mM sodium phosphate, pH7.7, 10mM DTT, 8mM MgCl₂, 4mM spermidine(HCl)₃, 0.5mM each of ATP, GTP and UTP, 12 μ M CTP and RNasin (1 unit/ μ l, Promega Biotech.). 50 μ Ci of α - 32 P CTP (800Ci/mmol, Amersham UK) and T7 RNA polymerase (10 μ g/ml, a kind gift of Bill Studier, Brookhaven) were used for each reaction, which were incubated at 37 $^{\circ}$ C for 60min. DNA template was removed by the addition of RNase-free DNase I (Promega Biotech., 1 unit/ μ g DNA) and incubation at 37 $^{\circ}$ C for 15 min. Samples were separated from unincorporated radioactivity on G100 sephadex columns and the appropriate fractions ethanol-precipitated with 10 μ g carrier tRNA. Each full-length RNA probe was purified by electrophoresis through a denaturing 6% polyacrylamide-7M urea gel. Position of the probe in the gel was determined by autoradiography (5 min. exposure) and the band was carefully excised. RNA was eluted in 2-3 volumes of 1X TE, 0.1% SDS, 1mM EDTA and Proteinase K (10 μ g/ml) at 37 $^{\circ}$ C overnight, then extracted with phenol twice, phenol/chloroform (1:1) and chloroform once and ethanol-precipitated with 10 μ g carrier tRNA.

RNase ("S1-type") transcript mapping.

The procedure used was modified from that of Melton *et al* (17). Total cellular RNA (50 μ g) was hybridised with the appropriate anti-sense RNA probe in 80% formamide, 40mM PIPES, pH6.4, 0.4M NaCl, 1mM EDTA at 45 $^{\circ}$ C overnight. Following hybridisation RNase A (40 μ g/ml) and RNase T1 (2 μ g/ml) were added and digestion allowed to proceed at 25 $^{\circ}$ C for 15 min (both enzymes from P.L.Biochemicals). RNase digestion was terminated by addition of SDS to 0.6% and Proteinase K (7mg/ml) followed by incubation at 37 $^{\circ}$ C for 15 min. The reaction was extracted once with

phenol/chloroform (1:1) and the ^{32}P -RNA/RNA hybrids ethanol-precipitated with 20 μg carrier tRNA. Hybrids were analysed by electrophoresis through a denaturing 6% polyacrylamide-7M urea gel and autoradiography at -70°C with intensifying screens.

DNA sequencing.

Nucleotide sequence of the pVU763-703 intergenic region was achieved by the Sanger dideoxy method from recombinant M13 bacteriophage (18).

RESULTS

Transcription termination separates the *hly* synthesis and secretion genes.

The hemolysin genes *hly C* and *hly A* dictate the synthesis of active intracellular hemolysin and the genes *hly B* and *hly D* its secretion. The genes *hly C*, *hly A*, *hly B* are transcribed coordinately from a region proximal to *hly C* (fig.1; 5,12,13) but the genes for synthesis and secretion are separated by an intergenic region containing an inverted repeat sequence capable of forming a stable stem-loop secondary structure (the most stable being $\Delta G=19.6\text{kcal/mol}$). This repeat is followed immediately by a run of thymidine residues, characteristic of a rho-independent transcription terminator (19). Both the palindrome and the poly-T sequence are, like the *hly* genes themselves, closely conserved in chromosomal *hly* determinants of *E.coli* (14,20) and also *Proteus* (fig. 1; $\Delta G=16.9\text{kcal/mol}$) indicating that they may be central to the pronounced polarity in the expression of the *hly C*, *hly A*, *hly B* transcription unit.

Figure 1. The *hly* determinant and the *hly A-hly B* intergenic sequence. Genes *hly A* and *hly C* encode synthesis and post-translational activation of hemolysin, genes *hly B* and *hly D* its specific secretion across both membranes. The previously reported inverted repeat and poly-T sequence within the *hly A-hly B* intergenic space of *E.coli* pBR202-312 (14) are shown in full and substitutions in the corresponding sequence of *P.vulgaris* 763-703 are indicated beneath it. Transcription of the *E.coli hly C*, *hly A* and *hly B* genes proceeds from a *hly C*-proximal promoter region as indicated by the arrows. The 3 T7-generated antisense RNA probes used in RNase mapping are depicted as RNA1 (*hly A*-internal), RNA 2 (spanning the intergenic region) and RNA 3 (*hly B*-internal).

An initial assessment of the putative role of the *hly A-hly B* intergenic region in transcription termination was made by introducing it, on the 477bp *Pvu* II-*Eco* RV restriction fragment, into the terminator-probe vector pKG1900 (16) between the *E.coli gal* promoter and *gal K* gene. In its natural orientation the insert reduced *gal K* expression over 5-fold (fig.2), a result typical of rho-independent terminators (21). There was also a 2-fold reduction in *gal K* expression when the intergenic sequence was inserted in the reverse orientation, possibly a result of the striking A-T rich sequence 5' to the inverted repeat sequence (fig.1) which could mimic the 3' poly-T sequence when inverted.

To demonstrate that *in vivo* intergenic termination was occurring at this locus we examined the levels of *hly* RNA generated by the plasmid pBR202-312, which bears the cloned 4-gene *hly* determinant of pHly152 (10,14) and, as a control, *E.coli* carrying the parent vector pBR322. Total cellular RNA was isolated from early logarithmic growth phase cultures of the *E.coli* strains and the pBR202-312 *hly A* transcript(s) terminating at, or elongating through, the putative *hly* transcription terminator were identified by RNase ("S1-type", ref.17) transcript mapping. This was achieved by probing the RNA with a purified, radioactively-labelled antisense RNA probe (RNA 2, fig.1) spanning the intergenic region, this having been produced at high levels *in vitro* from the reverse DNA sequence of the *Pvu* II-*Eco* RV restriction fragment inserted distal to the phage T7 promoter in plasmid T7-t(-). Following hybridisation, the lengths of the ³²P-labelled double-stranded RNA hybrids were defined by controlled digestion of the single strand RNA with RNases A and T1 and electrophoresis of the products through a 6% polyacrylamide-7M urea gel (fig.3, panel I). Autoradiography revealed that while no double-stranded RNA hybrid resulted from the control pBR322 assay, the *hly* plasmid pBR202-312 gave rise to 2 hybrids at 477bp and 180bp (i.e. 297bp shorter). The full length hybrid depicts transcripts reading through the intergenic region and into *hly B*, the shorter hybrid indicates transcripts terminating at a precise point within the probe sequence. The relative intensities of the 2 transcripts showed that in

Figure 2. *gal K* expression is reduced by the *hly* intergenic region. GalK levels encoded by pKG1900 with the 477bp *Pvu* II-*Eco* RV fragment spanning the *hly A-hly B* intergenic region interposed between the *gal* promoter and *gal K* (TAG denotes the presence of a translation stop codon in the upstream *gal E* reading frame). (+) and (-) indicate the normal and reverse *hly* orientation. Assays were blanked using parallel cultures of *E.coli* carrying pKO-1, which lacks promoter activity in front of *gal K* (16).

Figure 3. *In vivo* transcript termination within the *hly* intergenic region. RNA was isolated from *E. coli* 5K derivatives at A₆₀₀ 0.2 and hybridized to single-strand radioactively labelled antisense RNA spanning the intergenic sequence prior to separation by electrophoresis RNA hybrids (arrowed) derived from *E. coli* bearing (panel I) pBR202-312 and pBR322 vector, and (panel II) pKG1900 and pKG19-t(+); FP is the free probe. Panel III shows discrete termination of *in vitro* T7Ø10 transcription in pT7-t(+), lacking in pT7-t(-). Tracks M, pBR322 *Hpa* II marker restriction fragments

Figure 4. The calculated transcript termination point in the RNA sequence (22) of the *hly A-hly B* intergenic region.

pBR202-312 the majority of *hly C*, *hly A* transcripts were terminated in the intergenic region. The specificity of the *in vivo* termination point was confirmed (fig.3, panel II) by identical results from a parallel assay of RNA from *E.coli* strains carrying the *gal K* vector pKG1900 and its derivative pKG19-t(+). Further evidence of the termination function was obtained by the *in vitro* transcription of the *Pvu II-Eco RV* fragment from the T7 phage promoter $\phi 10$ by T7 RNA polymerase in the plasmid pT7-t(+) i.e. analogous to the generation of antisense RNA from the inverted sequence. The strong transcription directed by this T7-specific system was also arrested within the *hly A-hly B* intergenic region (fig.3 panel III).

The 297bp difference in length between the elongated and terminated transcripts, measured from fig.3 panel I, corresponds exactly to an *in vivo* transcript termination point within the poly-T sequence of the putative rho-independent terminator evident in the predicted RNA fold (ref.22, fig.4).

Antitermination dictates *hly B* expression and full hemolysin secretion.

The plasmid pBR202-312 carries ca.12.7kbp of pHly152 bearing the synthesis and secretion genes and also 1342bp *hly* -proximal DNA and ca.2060bp *hly*-distal DNA. This and other comparable *hly* recombinant DNAs (10,23,24) determine only weak specific secretion of active intracellular hemolysin protein and this is in contrast to the very efficient secretion of active hemolysin observed in wild-type hemolytic *E.coli* and *P.vulgaris* (10,23). Cloning of *hly* determinants carrying additional *hly* flanking sequence determines higher hemolytic activity (24) and in fig.5 we show that this is due primarily to a dramatic increase in specific secretion activity. The plasmid pEK50, which contains an additional ca.3460bp of *hly C*-proximal DNA (fig. 1) determines levels of extracellular hemolytic activity 20-fold higher than those directed by pBR202-312 (fig.5a). Intracellular

Figure 5. The effect of *E.coli* pHly152 *hly* promoter-upstream DNA. Levels of (a) intracellular and extracellular hemolytic activity (in hemolytic units), and (b) extracellular 110Kda hemolysin protein (arrowed) determined by early logarithmic phase cultures of *E.coli* 5KC carrying the *hly* recombinant DNAs pBR202-312 (-upstream region) and pEK50 (+upstream region) are presented with (c) the relative *in vivo* concentrations of *hly* A and *hly* B RNA in these cells, assayed by electrophoresis of hybrids formed with antisense RNA generated from the 792bp *Hin* dIII -*Eco* RI (RNA 1) and 812bp *Eco* RV-*Pst* I (RNA 3) fragments internal to these genes (fig. 1).

hemolytic activity is also higher, but to a much lesser extent (3-fold). The marked effect on secretion was shown vividly by direct assay of the extracellular cell-free 110Kda hemolysin protein (HlyA) secreted by the 2 strains (fig.5b).

That the activation of secretion function had a transcriptional basis was demonstrated by the quantitative comparison of *hly* transcripts from *E.coli* carrying the plasmids pBR202-312 and pEK50. Equivalent amounts of total RNA were isolated from the 2 strains at identical growth points and hybridised with each of 2 T7-generated anti-sense RNA probes of comparable length derived from the 792bp *Hin* dIII-*Eco* RI (RNA 1) and 812bp *Eco* RV-*Pst* I (RNA 3) restriction fragments which lie within the *hly* A and *hly* B genes, respectively (fig.1). The relative concentrations of *hly* A and *hly* B transcript generated by the 2 recombinant DNAs showed (fig.5c) that the additional *hly* C-proximal DNA of pEK50 increases the levels of transcript from both the *hly* A hemolysin structural gene (ca.3-fold, measured by scanning the hybrid bands in autoradiogrammes resulting from different exposure times) and, far more strikingly, from the *hly* B secretion gene (approximately

Figure 6. The *hly* promotor-upstream DNA suppresses intergenic termination. Hybridisation of antisense RNA 2 to total RNA isolated from *E.coli* 5KC carrying pBR202-312 (-) and pEK50 (+). RNA hybrids resulting from terminated and elongated transcripts are arrowed.

90-fold, again from scanning autoradiogrammes of different exposure times). The specific significance of this small increase in transcription of the synthesis gene *hly A* is unclear as using a control *bla* antisense RNA probe we find (data not shown) a parallel 2- to 3-fold increase in the level of pBR322 vector β -lactamase gene transcript, resulting in a 2.5 fold increase in ampicillin resistance of *E.coli* 5KC. What is nevertheless clear is that the drastic enhancement of secretion function is due to a dramatic increase in transcription of the secretion gene *hly B*.

To ascertain the basis for the markedly preferential increase in *hly B* transcription, qualitative differences in *hly* transcription were assessed by probing the same RNAs generated by pBR202-312 and pEK50 with RNA 2, complementary to the *hly A-hly B* intergenic region (fig.6). RNA 2 hybridisation confirmed that no additional transcript was initiated within the *hlyA-hlyB* intergenic space and showed that the substantial activation of *hly B* expression has 2 components, a small increase in transcription through *hly A* and, of much greater prominence, transcript elongation through the intergenic region distal to *hly A*. The data show that pEK50, containing the *hlyC*-proximal DNA, directs a drastic reduction in the transcription termination exerted by the *hly A-hly B* intergenic terminator. The relative hybridisation intensities determined by the terminated and elongated transcripts within each track again showed that ca.80% of *hly* transcripts made by pBR202-312 were terminated distal to *hly A* but that in contrast practically all (i.e. over 98%) of

the *hly* transcripts directed by pEK50 were elongated into *hly* B. The difference in hybridisation intensity evident between the 2 tracks would be expected to be ca.8-fold, resulting from the ca.3-fold increase in *hly* A (RNA I) hybridisation (fig.5) and the 2.6-fold greater length (and thus radioactivity) of the elongated hybrid. The apparently greater disparity seen in fig.6 may indicate inherent differences between the RNA 2 hybridisations. Notwithstanding this, the data indicate strongly that the preferential increase in *hly* B transcript levels was due primarily to transcript elongation exerted through the *hly* A-*hly* B intergenic rho-independent transcription terminator.

DISCUSSION

The sequence and organisation of the 4 contiguous hemolysin genes encoding hemolysin synthesis and secretion are closely conserved among *E.coli* and the related enterobacteria *Proteus* and *Morganella* (9,10,12,23,25). The secretion of the cytotoxic hemolysin protein is remarkable in that it proceeds without N-terminal signal recognition or peptide cleavage and dictates export across both membranes out into the medium (1,2,9). The export is dependent upon an as yet uncharacterised C-terminal signal (9,26) and apparently requires only 2 specific membrane proteins encoded by the export genes *hly* B and *hly* D (8,9,23), at least the first of which is co-transcribed with those for synthesis (3,4,11). The *hly* determinant therefore presents a defined example of coordinated expression of synthesis and secretion of protein and it would not be surprising that a possibility exists for modulating the relative expression of the 2 functions. Our data indicate that this is achieved by transcription antitermination.

The genes for synthesis of active intracellular hemolysin immediately precede those encoding its subsequent export and are separated in the wild-type conjugable plasmid pHly152 by a 60bp intergenic region which contains an intergenic inverted repeat sequence. While extragenic palindromic (REP) sequences can affect *E.coli* gene expression by stabilizing upstream mRNA (27), the *hly* inverted repeat immediately precedes a run of thymidine residues (14) which is characteristic of a rho-independent transcription terminator (19,21) and is conserved in chromosomal *hly* determinants of *E.coli* (20) and *P.vulgaris* (this paper). The capacity of the *hly* A-*hly* B intergenic sequence to function as a transcription terminator was demonstrated by its insertion between the *gal* promoter and the *gal* K gene which reduced *in vivo* expression of the latter by ca.80%. The use of antisense RNA spanning the intergenic region demonstrated that this was due to transcript termination within the poly-U sequence. This was confirmed with parallel probing of RNA from *E.coli* expressing the 4-gene *hly* determinant on a multicopy recombinant DNA. In this case, ca.80% of all transcripts reading through the *hly* synthesis genes were terminated specifically within the poly-U sequence. Specific transcript termination was also evident *in vitro* during transcription of the intergenic sequence from the phage T7 ϕ 10 promoter by T7 RNA polymerase.

Basal expression of the specific secretion gene *hly* B resulting from the early termination was elevated to wild-type levels by additional cloning of the contiguous ca.3.5 kbp DNA upstream of the

hly operon promotor region. This upstream DNA acted to increase preferentially hemolysin secretion and this was dictated by a drastic elevation (measured approximately as 90-fold) in the level of *hly* B mRNA. This was shown by direct assay of *hly* mRNA species to result primarily from suppression of specific *in vivo* intergenic termination from 80% to 1%. Virtually all *hly* transcripts elongated through the intergenic region into *hly* B.

Inverted repeat sequences in bacteria routinely define specific 3' termini of monocistronic and polycistronic messages (19,21). The exploitation of transcript termination as a means of enhancing operon polarity is less frequent and only a few examples of these have as yet been shown to be regulated. Regulation is positive, dictates transcript elongation through the termination point, and is termed transcription antitermination (28). Anti-terminator activity has been described in the rRNA operon (29,30) and also the *tna* (31) and *bgl* (32) operons required for the catabolism of tryptophan and β -glucosides, all in *E.coli*. In addition it has been suggested that expression of the tryptophan biosynthetic operon in *Bacillus subtilis* involves anti-termination via inactivation of a negative regulator (33). Anti-termination has also been described recently in the eukaryotic proto-oncogenes *c-myb* and *c-myc* and also the HIV-1 LTR (34,35,36) but as with the prokaryotic examples the mechanism of transcript elongation has not been defined.

Identification of the factors effecting antitermination in bacterial systems has been possible only in the case of the *bgl* operon where one of the products of the operon itself, *bgl* C, acts as a positive regulator to elongate operon transcripts (32). Antitermination in the rRNA operons is dictated by an unidentified chromosomal locus (30). Our data show that anti-termination within the pHly152 *hly* operon is effected by a closely-linked upstream factor and indicate that this is accompanied by an increase in transcription upstream of the transcription terminator. Primer-extension experiments support the view that the latter results, only in *cis*, from increased transcript initiation in the natural *hly* operon promoters (Koronakis, unpublished data), but we do not yet know whether the increase in transcription initiation is specific to the *hly* region nor whether it and the more prominent transcript elongation are mechanistically connected. Precise identification of the *hly* C-proximal factor(s) influencing transcription initiation and antitermination in the *hly* operon will allow one to address these questions and also assess the role of the potentially flexible transcript termination and elongation in coordinating the hemolysin synthesis and secretion functions.

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REFERENCES

1. Haertlein M, Schiessl S, Wagner W, Rdest U, Kreft J and Goebel W (1983) J. Cell. Biochem. 22:87-97
2. Felmlee T, Pellett S and Welch RA (1985a) J Bacteriol. 163:94-105.
3. Juarez A, Haertlein M and Goebel W (1984a) J Bacteriol. 160: 161-168.
4. Juarez A, Hughes C, Vogel M and Goebel W (1984b) Mol. Gen. Genet. 197: 196-203.
5. Nicaud JM, Mackman N, Gray L and Holland IB (1985a) Mol. Gen. Genet. 199:111-116.

6. Gerlach JH, Endicott J, Juranka P, Henderson G, Sarangi F, Deuchars K and Ling V. (1986) *Nature* 324:485-489.
7. Gros SP, Croop J and Housman D (1986) *Cell* 47:371-380.
8. Wagner W, Vogel M and Goebel W (1983) *J. Bacteriol.* 154:200-210.
9. Mackman N, Nicaud JM, Gray L and Holland IB (1986) *Curr. Top. Micro. Immun.* 125: 159- 181.
10. Hacker J and Hughes C (1985) *Curr. Top. Micro. Immun.* 118:139-162.
11. Mackman N, Nicaud JM, Gray L and Holland IB (1985) *Mol. Gen. Genet.* 201:282-288.
12. Koronakis V and Hughes C (1988) *Mol. Gen. Genet.* in press
13. Nicaud JM, Mackman N, Gray L and Holland IB (1985b) *FEBS Lett.* 187:339-334.
14. Hess J, Wels W, Vogel M and Goebel W (1986) *FEMS Microbiol. Lett.* 34:1-11.
15. Zabala JC, Garcia-Lobo JM, Diaz-Aroca E, dela Cruz F and Ortiz JM (1984) *Mol. Gen. Genet.* 197:90-97.
16. McKenney K, Shimatake H, Court D, Schmeissner U, Brady C and Rosenberg M (1981) In *Gene amplification and analysis II* (Eds. Chirikjian JS and Papas TS) Elsevier NY, USA, pp383-415.
17. Melton DA, Krieg PA, Rebagliati MR, Maniatis T, Zinn K and Green MR (1984) *Nucl. Acids Res.* 12:7035-7056.
18. Sanger F, Nicklen S and Coulson AR (1977) *Proc. Natl. Acad. Sci. USA* 74:5463-5467.
19. Holmes WM, Platt T and Rosenberg M (1983) *Cell* 32: 1029-1032.
20. Felmlee T, Pellett S, Lee E-Y and Welch RA (1985b) *J. Bacteriol.* 163:88-93.
21. Rosenberg M, Chepelinsky AB and McKenny K (1983) *Science* 22: 734-739.
22. Zuker M and Stiegler P (1981) *Nucl. Acids Res.* 9: 133-148.
23. Koronakis V, Cross M, Koronakis E, Senior B and Hughes C (1987) *J. Bacteriol.* 169:1509-1515
24. Gonzalez-Carrero M, Zabala JC, dela Cruz F and Ortiz J (1985) *Mol. Gen. Genet.* 199:106-110
25. Muller D, Hughes C and Goebel W (1983) *J. Bacteriol.* 153:846-851.
26. Mackman N, Baker K, Gray L, Haigh R, Nicaud JM and Holland IB (1987) *EMBO J* 6: 2835-2842.
27. Newbury SF, Smith NH, Robinson EC, Hiles ID and Higgins CF (1987) *Cell* 48:297-310.
28. Roberts JW (1988) *Cell* 52: 5-6.
29. Li SC, Squires CL and Squires C (1984) *Cell* 38:851-860.
30. Morgan EA (1986) *J. Bacteriol.* 168:1-5.
31. Stewart V and Yanofsky C (1985) *J. Bacteriol.* 164: 731-740.
32. Mahadevan S and Wright A (1987) *Cell* 50:485-494.
33. Shimotsu H, Kuroda M, Yanofsky C and Henner D (1986) *J. Bacteriol.* 166:461-471.
34. Bender TP, Thompson CB and Kuehl M (1987) *Science* 237: 1473-1476.
35. Bentley DL and Groudine M (1986) *Nature* 321: 702-706.
36. Kao S-Y, Calman AF, Luciw PA and Peterlin BM (1987) *Nature* 330: 489-493.