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Convergently Functional, Rho-Independent Terminator in *Salmonella typhimurium*

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A typical Rho-independent terminator of transcription was found at the end of the histidine operon of *Salmonella typhimurium*. This site is used to terminate, in addition to the *his* operon mRNA, a 1,200-nucleotide RNA of unknown function transcribed on the opposite strand. The efficiency of termination of transcription at this site was investigated in vivo and in vitro by cloning of the terminator structure in either orientation in vector systems used to study regulatory signals. Termination of transcription at this site was very efficient, both in vivo and in vitro, and in both orientations.

Termination of transcription in bacteria and phage is an intricate process to which much attention has been paid. Two classes of termination sites have been identified. The first one comprises the so-called Rho-independent terminators, which share common structural features (a G-C-rich inverted repeat followed by a run of thymidylate residues) and do not require the presence of cellular factors. The second class, known as factor-dependent terminators, does not exhibit a consensus sequence and requires protein factors for proper function (1, 15, 27).

An increasing body of evidence indicates that termination of transcription is involved in important regulatory functions. These include regulation of expression of downstream genes as in the case of attenuation (2, 17, 18, 35) or of upstream genes, by controlling the rate of mRNA degradation (retroregulation), as in the case of the λ*int* gene expression (29).

In the course of studies on the structural organization of the distal portion of the histidine operon (5, 13, 14), we identified a typical Rho-independent terminator at the end of the operon in *Salmonella typhimurium* (5). In this paper, we present evidence that the *his* terminator is also used in vivo to terminate RNA molecules synthesized on the opposite strand. In addition, we cloned DNA fragments containing this site into vector plasmids containing different *Escherichia coli* K-12 promoters and measured the relative in vivo and in vitro efficiencies of termination in either orientation.

MATERIALS AND METHODS

Bacterial strains. The following strains were used: *S. typhimurium* LT2 (wild type), FB281 (*hisR1203*); *E. coli* FB1 Δ(*hisGDCBHAFIE750*) *gnd rhaA* and N100 (*galK2 recA13*).

Plasmids. Plasmid pHS10000 contains the entire *S. typhimurium his* operon and flanking regions in a *Pst*I fragment of 10,000 base pairs (bp) cloned in vector pBR322. pHS9750 contains the same fragment but also carries the *hisFI135* deletion. Plasmids pKL600 and pKO1 were a gift of K. McKenney, National Institutes of Health. Plasmid pKL600 was used to clone the *his* terminator in either orientation. The 110-bp *Sau*3AI-*Hin*dIII fragment, containing the *his* terminator (Fig. 1), was purified and the 3′ recessed termini were filled with the Klenow fragment of *E. coli* DNA polymerase I as described previously (19). The fragment was inserted by blunt end ligation in the unique *Sma*I site of pKL600. The orientation was verified with respect to the *Pvu*I site which is generated at the *Sma*I-*Sau*3AI border. The resulting plasmids, containing the fragment cloned in either orientation, were named pKSH110 and pKHS110.

A 375-bp *Alu*I fragment containing the *E. coli* K-12 *hisGp* promoter and the attenuator (34) was cloned in the *Sma*I site of vector pKO1 (21) by blunt end ligation, and the proper orientation was verified with respect to the unique *Bgl*II site present in the cloned fragment and the unique *Hin*dIII site present in the vector (see Fig. 6). The resulting plasmid pAR1 was used to clone the *his* terminator in either orientation. The 175-bp *Sau*3AI fragment, containing the *his* terminator (Fig. 1), was purified and inserted in the unique *Bgl*II site of pAR1. The orientation was verified with respect to the asymmetric *Hin*dIII site present in the cloned *Sau*3AI fragment. The resulting plasmids were named pAR15 and pAR16.

Plasmid DNA was purified by the method of Clewell (7).

Plasmid copy number was determined by dot blot hybridization (32), using nick-translated pBR322 DNA as a probe.

The assay for galactokinase in strain N100 harboring the pKHS110 and pKSH110 plasmids was performed as described elsewhere (21).

Purification, labeling, and sequencing of DNA fragments. DNA fragments or strands were isolated through acrylamide slab gels and recovered by electroelution as described previously (19).

5′ End labeling with polynucleotide kinase was performed as described by Maxam and Gilbert (20). 3′ End labeling was performed with reverse transcriptase (12) or with Klenow fragment of DNA polymerase (3). Nick translation was performed by the method of Rigby et al. (25).

DNA sequence determination was by the method of Maxam and Gilbert (20).

RNA preparation and S1 mapping. Total bacterial RNA was extracted by the guanidine hydrochloride procedure previously described (13). RNA-DNA hybridization, S1 nuclease digestion, and analysis of the hybrids on polyacrylamide denaturing gels were performed as described

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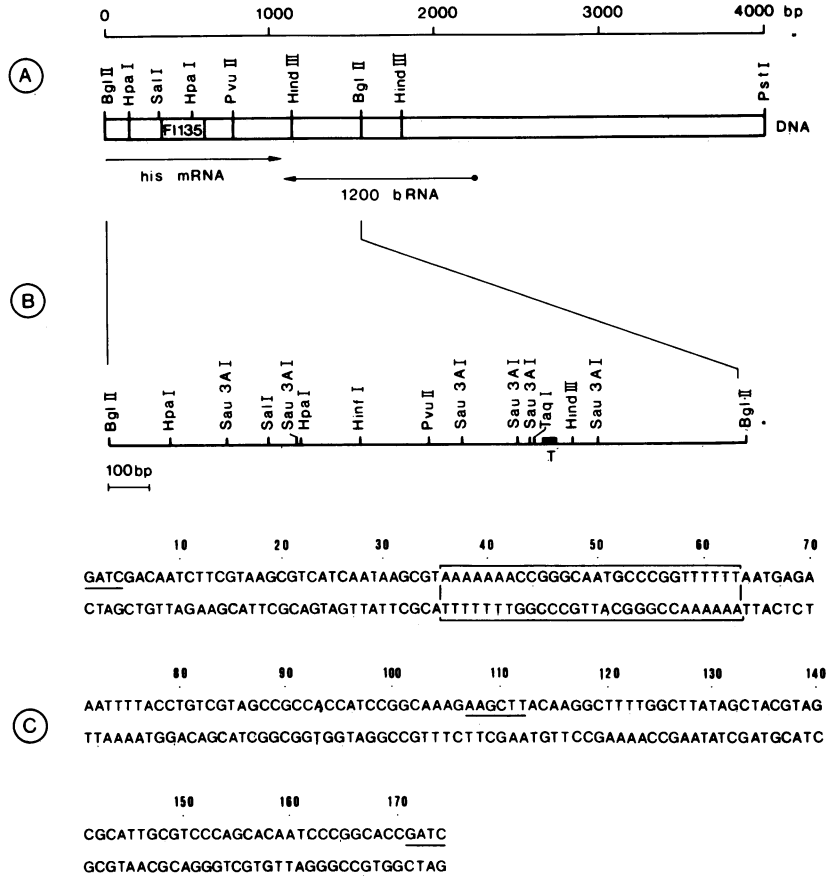

FIG. 1. Physical and restriction map of the distal region of the *S. typhimurium his* operon. (A) The location of the *hisFI135* deletion and some of the restriction sites utilized in this study are indicated for the most distal part of the *his* operon and downstream region. Also shown are the length and direction of the transcripts synthesized from this region of DNA (arrows). (B) Detailed restriction map of the 1,550-bp *Bgl*II fragment containing the *his* terminator (T) and used for the low-resolution S1 mapping experiments. (C) Nucleotide sequence of the *his* terminator and flanking regions. The palindromic structure is boxed. The *Sau*3AI sites and the *Hin*dIII site are underlined. Each region of the sequence was determined more than once and on both strands.

elsewhere (9). The reaction mixtures contained different amounts of labeled DNA probes and different amounts of specific RNA which are specified below and in the figure legends.

Analysis of S1 nuclease mapping experiments. When double-stranded DNA probes are used for S1 mapping, one problem often encountered is competition of homologous DNA with RNA in annealing to the radioactive DNA probe. Since in 80% formamide, DNA-RNA hybrids are more stable than the DNA duplex (6), we chose hybridization conditions stringent enough to overcome such a problem. Between 10 and 100 ng of the probe was hybridized to 2.5 to 50 μg of total RNA for 3 h at 53°C in 10-μl volumes. Subsequent treatment with S1 nuclease (type III; Sigma Chemical Co.) was for 1 h at 37°C (100 U for 300-μl reactions). We routinely checked that the probe did not reanneal on itself by performing hybridizations of the probe alone or with etherologous RNA. In every case, we observed full sensitivity to S1 nuclease. For the high-resolution S1 mapping experiments, 100 ng of single stranded, 3'-labeled DNA probe and 100 μg of total RNA were denatured together at 75°C, and the solution was allowed to cool to 37°C over a period of 6 h before S1 nuclease treatment of hybrids (4).

Northern blot analysis. Total RNA from strain FB281 (*hisR1203*) was fractionated on 1% agarose gels containing formaldehyde (11) as modified by Maniatis et al. (19). RNA transfer to nitrocellulose filters and hybridization with ^{32}P-labeled fragments were performed by the method of Thomas (32).

In vitro transcription. Supercoiled plasmid DNAs (60 μg/ml) or mixtures of DNA fragments (30 μg/ml) were transcribed in vitro as previously described (10). The RNA synthesis mixture was analyzed on 6% acrylamide denaturing gels (28).

RESULTS

Distal region of the *S. typhimurium his* operon and the location of the 3' ends of transcripts. We have recently cloned several DNA fragments containing the *his* operon of *S. typhimurium* in plasmid vectors and have mapped the 3' end of the *his* operon transcript (5). The *his* mRNA terminates within a 1,550-bp *Bgl*II fragment to the immediate left of a *Hin*dIII site (Fig. 1A). To study in more detail the process of transcription termination at the end of the *his* operon, we established a fine restriction map of the distal region of the operon (Fig. 1B). The sequence of the DNA region containing the putative terminator site was determined (Fig. 1C). This region contains a palindromic structure that exhibits the typical characteristics of a Rho-independent terminator site. In addition, this sequence possesses a peculiar feature: the palindrome is flanked by a run of thymidylate residues on both strands. Termination of transcription at this site was

investigated by low-resolution S1 mapping. The 1,550-bp *Bgl*II fragment (Fig. 1B) was purified, labeled at the 3′ ends, and hybridized with total RNA isolated from several strains (see above). Hybridization was first performed with RNA isolated from strain FB1 (a complete *his* deletion) harboring plasmid pHS10000 which contains the entire *his* operon and flanking regions. Mapping from *Bgl*II disclosed two major protected fragments of 1,100 and 450 nucleotides (nt) (Fig. 2, lanes 1 to 3). The presence of a second protected hybrid suggests either that another transcription stop site exists on the same strand or that an RNA is transcribed on the opposite strand and is terminated at approximately the same site.

To discriminate between these two possibilities, we hybridized the 1,550-bp *Bgl*II fragment to the RNA extracted from strain FB1 harboring plasmid pHS9750 which contains the same *Pst*I fragment cloned in pHS10000 derived from a strain carrying the *hisFI135* deletion (Fig. 1A). The 1,100-nt, *his*-specific protected fragment was reduced to 350 nt, whereas the 450-nt fragment was not affected (Fig. 2, lane 6). This result indicates that the 450-nt fragment hybridizes to an RNA transcribed toward the *his* operon. Further experimental evidence was obtained by cleaving the labeled 1,550-bp *Bgl*II fragment with several restriction enzymes before hybridization. Secondary cleavage with *Pvu*II generated two single 3′-labeled fragments of similar size (775 bp). The 1,100-nt protected fragment was now reduced to 775 nt, whereas the 450-nt one was unaffected (data not shown). Moreover, when the 1,550-bp *Bgl*II fragment was cleaved with *Hpa*I, which generates two 3′-labeled fragments (one proximal of 150 bp and another distal of 1,150 bp), the 1,100-nt protected fragment was reduced to 150 nt and the 450-nt fragment was unaffected (data not shown), as expected from the restriction map (Fig. 1B). In summary, these experiments demonstrate the existence of two transcripts that are convergently synthesized on opposite strands and are apparently terminated at the single Rho-independent site.

More precise mapping of the 3′ ends of the convergent transcripts. To define precisely the 3′ ends of the transcripts, we conducted two different kinds of experiments. To demonstrate that the 3′ ends of the RNAs do not extend beyond the terminator structure, we measured the resistance of RNA-DNA hybrids to the action of S1 nuclease (S1 protection). From the DNA sequence, we could deduce which were the *his* and anti-*his* sense strands. A 150-bp *Sau*3AI fragment proximal to the terminator and a 410-bp *Hin*dIII-*Bgl*II fragment distal to the terminator (Fig. 1B) were labeled at the 3′ ends, and the strands were separated. The separated strands were hybridized to RNA isolated from strain FB1 harboring plasmid pHS10000 and were subjected to S1 nuclease digestion. With the 150-bp *Sau*3AI fragment, only the strand complementary to the *his* RNA was protected. On the contrary, with the 410-bp *Hin*dIII-*Bgl*II fragment, only the strand complementary to the *his*-convergent RNA was protected (data not shown). This result proves that the *his* RNA does not extend beyond the *Hin*dIII site located 40 bp from the palindrome; the *his*-convergent RNA does not extend beyond the *Sau*3AI site located 50 bp from the palindrome.

To identify the 3′ nucleotides of the transcripts, we performed a high-resolution S1 mapping of the terminator region. A 175-bp *Sau*3AI fragment, spanning the terminator (Fig. 1B), was labeled at the 3′ ends, and the strands were separated. Each strand was hybridized to RNA isolated from strain FB1 harboring plasmid pHS10000, and the hybrids were digested with S1 nuclease. The same labeled strands were sequenced, and the products were run, together with the S1-digested hybrids, on a 10% acrylamide slab gel to generate markers. The results obtained with the 3′-labeled coding strand for the *his*-convergent RNA are shown in Fig. 3. As expected, the majority of the transcripts terminated downstream from the secondary structure stem and loop within the run of uridylate residues. In addition, protected hybrids were observed coincident with the nucleotides corresponding to the loop of the palindrome. We believe that these protected fragments do not represent terminated RNA molecules but are due to intrastrand pairing of the DNA probe resulting in S1 sensitivity of the single-strand looped regions. In the parallel experiment, using the 3′-labeled coding strand for the *his* RNA, no protection of the DNA probe was observed. The putative region of homology between the DNA strand and the *his* RNA (about 60 nt) is probably too short to generate stable hybrids.

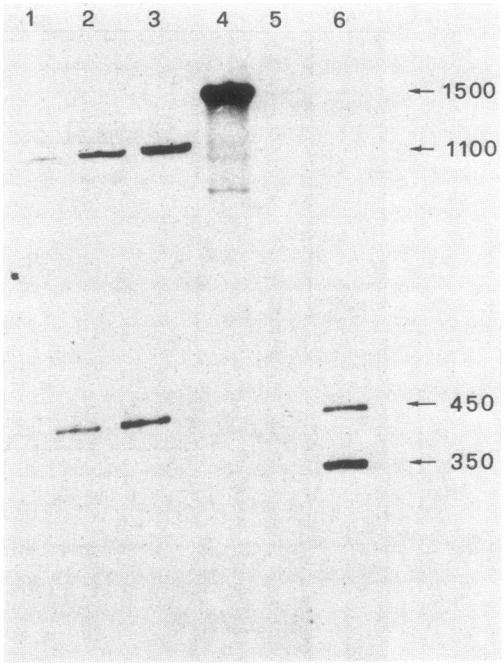

FIG. 2. Localization of the 3′ ends of the transcripts at the *his* terminator by S1 nuclease gel mapping. The 1,550-bp *Bgl*II fragment (Fig. 1B) was labeled at the 3′ ends (lane 4) and hybridized to 5, 12.5, and 25 μg of total RNA (lanes 1, 2, and 3, respectively) extracted from strain FB1 harboring plasmid pHS10000. The hybrids were digested with S1 nuclease and analyzed on 6% acrylamide denaturing gels. The same fragment was also hybridized to 25 μg of total RNA extracted from strain FB1 harboring plasmid pHS9750 (*hisFI135* deletion) (lane 6). Lane 5: same DNA probe hybridized to 25 μg of total RNA from strain FB1 and digested with S1 nuclease.

RNA transcribed in opposite direction is present in wild-type cells. Most of the S1 mapping experiments reported in the previous sections were performed with RNA extracted from strain FB1 harboring plasmid pHS10000. We wanted to prove that transcription in the opposite direction reflected the presence of a bacterial promoter and was not due to read-through transcription from promoters located in the vector sequences (31). Total RNA was isolated from an *S. typhimurium* strain (FB281 *hisR1203*) constitutive for *his* operon expression and lacking plasmids. This RNA was hybridized to the 1,550-bp *Bgl*II fragment labeled at the 3′ ends, and the hybrids were digested with S1 nuclease.

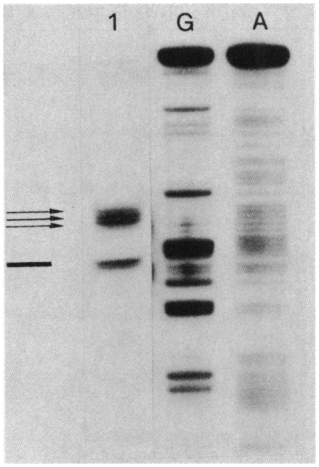

FIG. 3. High-resolution S1 nuclease mapping of the *his*-convergent RNA 3′ end. Mapping was performed with the 3′-end-labeled, 175-bp, *Sau*3AI fragment. The strands were separated, and the coding strand for the *his*-convergent RNA (100 ng) was hybridized to 100 μg of total RNA as described in the text. A partial sequence ladder of the DNA probe (G, A) is shown beside the nuclease-resistant DNA (1). The 3′ end of the *his*-convergent RNA is heterogeneous, terminating with different uridylate residues corresponding to the end of the palindrome (arrows). The other protected hybrid (bold line) coincides with the nucleotides constituting the loop of the palindrome.

Again, both the 1,100- and the 450-nt protected fragments were present, although the *his*-specific band was much more prominent (Fig. 4A). This result establishes that the RNA transcribed in opposite direction with respect to the *his* operon is expressed in the wild-type cells. We therefore tried to localize the 5′ end of the *his*-convergent transcript by low-resolution S1 mapping. The 2,300-bp *Bgl*II-*Pst*I fragment (Fig. 1B) was 5′ labeled at the *Bgl*II site, hybridized to total RNA from strain FB281, and digested with S1 nuclease. A single protected fragment of 750 nt was observed (Fig. 4B). This result suggests that a transcription initiation site is present on the opposite strand with respect to the *his* operon and gives an approximate estimate of the size of the *his*-convergent RNA being transcribed (1,200 nt). Independent confirmation of these results was obtained by performing a Northern blot analysis of total RNA isolated from *S. typhimurium*. The RNA was fractionated on 1% agarose gel, transferred to nitrocellulose filters, and hybridized to the 410-bp *Hin*dIII-*Bgl*II fragment (Fig. 1B) which contains sequences distal to the terminator. A single 1,200-nt RNA molecule was detected (Fig. 5). Hybridization with a different DNA probe containing regions on both sides of the terminator showed, in addition to the 1,200-nt RNA, the presence of *his*-specific transcripts (data not shown). These data confirm the existence and the size of the RNA convergently transcribed on the opposite strand.

Cloning and fusion of the *his* terminator to the gene for galactokinase and galactokinase expression in chimeric plasmids. The function of the *his* terminator in vivo was studied by inserting this region of DNA in the plasmid vector pKL600 constructed and kindly provided by K. McKenney. In this vector, the *galK* gene has been inserted into a pBR322 derivative harboring the *lac* promoter. The presence of various unique restriction sites allows easy insertion of any DNA fragment between the *lac* promoter and the *galK* gene. The 110-bp *Sau*3AI-*Hin*dIII fragment containing the *his* terminator was cloned in either orientation in the pKL600 vector (see above). The resulting plasmids were named pKSH110 and pKHS110. The levels of galactokinase were measured in strain N100 harboring the plasmids pKL600,

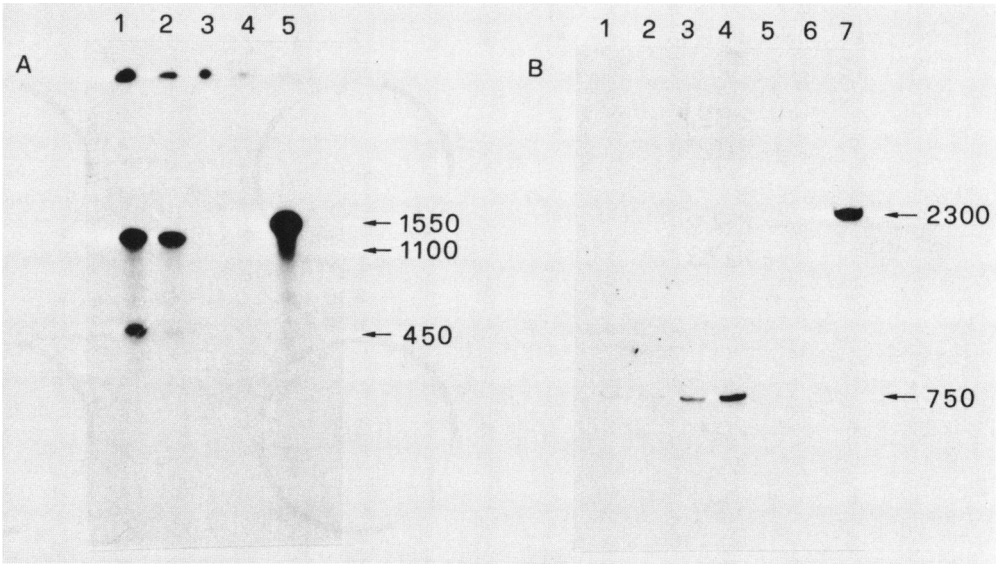

FIG. 4. S1 mapping of the 3′ ends of the transcripts and of the 5′ end of the *his*-convergent transcript with RNA extracted from wild-type *S. typhimurium* cells. (A) The 1,550-bp *Bgl*II fragment, labeled at the 3′ end (lane 5), was hybridized to 25 μg of total RNA extracted from strain FB1 harboring plasmid pHS10000 (lane 1) or to 25 μg of total RNA extracted from strain FB281 (*hisR1203*) (lane 2) and digested with S1 nuclease. The probe was also hybridized to 25 μg of RNA from strain FB1 (lane 3) and to itself (lane 4) and digested with S1 nuclease. (B) The 2,300-bp *Bgl*II-*Pst*I distal fragment (Fig. 1A) was labeled at the 5′ ends (lane 7), hybridized to 5, 12.5, 25, and 50 μg (lanes 1, 2, 3, and 4, respectively) of total RNA extracted from strain FB281 (*hisR1203*), and digested with S1 nuclease. The probe was also hybridized to 50 μg of FB1 RNA (lane 5) and to itself (lane 6) and digested with S1 nuclease. Labeled fragments of known length were used as internal markers.

pKSH110, and pKHS110, as described above. In pKL600, galactokinase was expressed rather efficiently in both rich and minimal media (Table 1). The presence of a terminator structure in proper orientation between the *lac* promoter and the *galK* gene is expected to reduce galactokinase levels. Such reduction in *galK* expression is a direct measure of terminator efficiency (26). When the terminator was oriented so that it functioned to terminate the *his* mRNA (plasmid pKSH110), its efficiency was calculated to be 93%. In the opposite orientation (plasmid pKHS110), the efficiency is possibly even higher (96%). Similar values were found under different growth conditions. The plasmid copy number (see above) was found not to vary appreciably among the various plasmids used and in strains grown under similar growth conditions.

Cloning and in vitro function of the *his* terminator in either orientation. The in vivo data indicate that the *his* terminator functions very efficiently in either orientation. In vivo it is not possible to distinguish whether the 3' end of a transcript is the result of true termination at a site or is due to processing of longer transcripts. Moreover, in vivo and in vitro data on several terminators often show marked differences in efficiency, as well as in the overall mechanism by which the termination process is regulated. To analyze the behavior of the *his* terminator in an in vitro transcription system, we cloned the terminator structure between the primary *hisGp* promoter and the *his* attenuator. From previous in vitro studies, we know that the *hisGp* promoter functions very efficiently (34) and that termination of transcription at the *his* attenuator is about 100% efficient (10). The *his* operon regulatory region was cloned in vector plasmid pKO1 (see above). The resulting plasmid pAR1 was used to clone the *his* terminator in either orientation. The two plasmids were named pAR15 and pAR16 (Fig. 6). In vitro transcription was performed as detailed above. One such experiment is shown in Fig. 7A. pAR1 template (lane 3) generated a single 180-nt transcript which represents the *his* leader RNA (10, 34). Both pAR15 (lane 7) and pAR16 (lane 5) plasmids directed the synthesis of a 360-nt RNA which again represents the RNA terminated at the *his* attenuator. In addition, pAR15 synthesized a 280-nt RNA and pAR16 synthesized a 185-nt RNA which represent transcripts initi-

TABLE 1. In vivo and in vitro efficiency of the *his* terminator

Plasmid[a]	Medium[b]	No. of *GalK* units[c]	Efficiency (%)	
			his terminator	*his* attenuator
In vivo				
pKL600	LB	732	0	
	Min	942		
pKHS110	LB	28	96	
	Min	33		
pKSH110	LB	40	93	
	Min	76		
In vitro[d]				
pAR1				100
pAR15			74	26
pAR16			32	68

[a] In plasmids pKSH110 and pAR16 the *his* terminator is cloned in the *his* orientation.
[b] LB, Rich medium; Min, minimal medium.
[c] The data are averages of three experiments.
[d] The bands corresponding to the different transcripts were excised from the gels and counted. Data are the averages of two experiments.

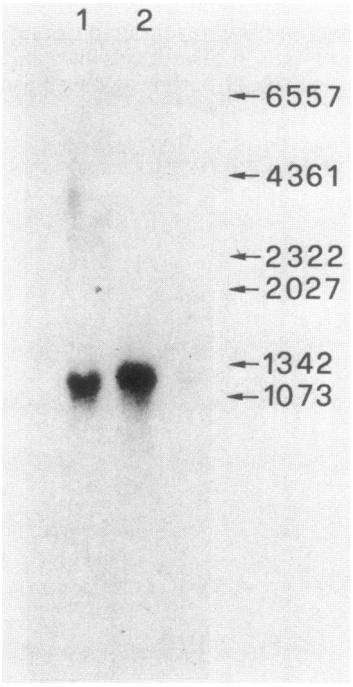

FIG. 5. Northern blot analysis of total RNA extracted from strain FB281 (*hisR1203*). Samples (10 and 20 μg) of total RNA (lanes 1 and 2, respectively) were sized on 1% agarose gel, transferred to nitrocellulose filters, hybridized to nick-translated DNA (specific activity, 10^8 cpm/μg, 10^6 cpm/ml) for 16 h at 42°C, and exposed for 24 h. The RNA was hybridized to the 410-bp *Hin*dIII-*Bgl*II fragment (Fig. 1B) comprising regions distal to the terminator. Markers used in the gel were λ DNA digested with *Hin*dIII and φX174 DNA digested with *Hae*III.

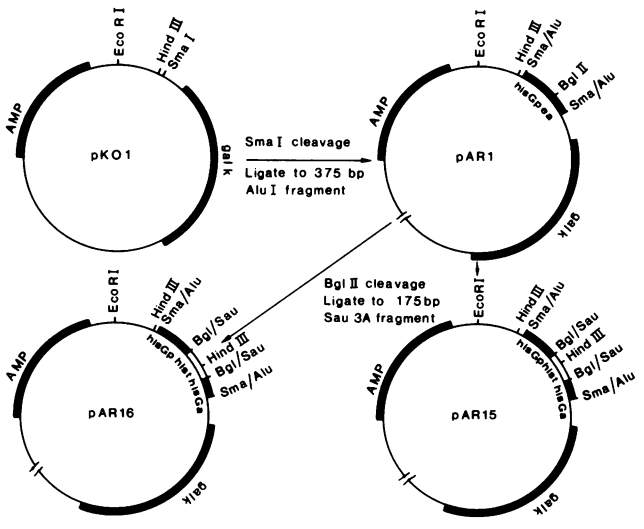

FIG. 6. Vector plasmid pKO1 and derivative (pAR1, pAR15, and pAR16) used to study the function of the *his* terminator in vitro. The circular map of pKO1, approximately to scale, shows some of the restriction sites, including the *Sma*I site, used to clone the 375-bp *Alu*I fragment and the position of the AMP resistance and *galK* genes. In pAR1, the fragment containing the *hisGp* promoter, leader, and attenuator (hisGpea) is shown, together with the *Bgl*II site used to clone the *his* terminator (hist) in the his orientation (pAR16) and in the opposite orientation (pAR15).

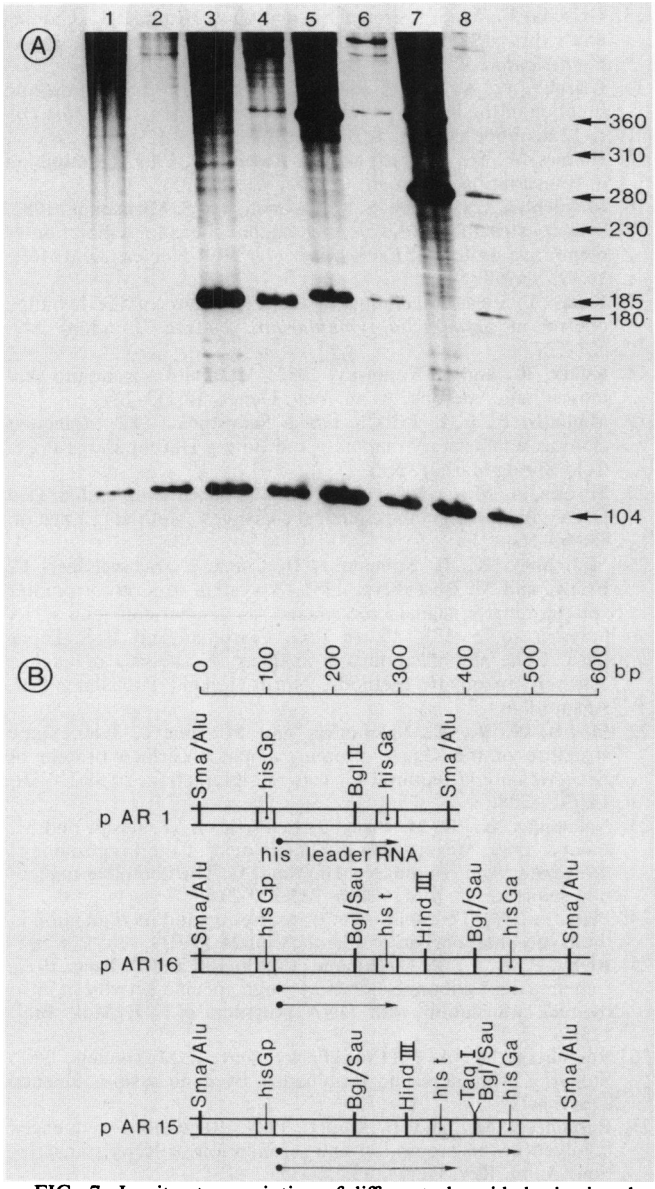

FIG. 7. In vitro transcription of different plasmids harboring the *his* terminator in either orientation. (A) pBR322 supercoiled DNA (lane 1) linearized with *Hin*dIII (lane 2); pAR1 supercoiled DNA (lane 3) linearized with *Hin*dIII (lane 4); pAR16 supercoiled DNA (lane 5) linearized with *Hin*dIII (lane 6); pAR15 supercoiled DNA (lane 7) cleaved with *Taq*I (lane 8). Faster-migrating transcripts in this lane are vector specific since they were also observed when pAR1 DNA was transcribed after cleavage with *Taq*I (data not shown). (B) Schematic diagram of the cloned DNA fragments and of the transcripts generated by pAR1, pAR16, and pAR15.

ated at the *hisGp* promoter and terminated at the *his* terminator in the two different orientations. All of the plasmids also directed the synthesis of the 104-nt vector-specific transcript (34). A transcriptional map of the different plasmids, together with the location of the regulatory signals in the DNA inserts, is shown in Fig. 7B. Assuming termination at the *his* attenuator to be 100% efficient, it was found that the *his* terminator functioned with a 32% efficiency when in the *his* orientation and with a 74% efficiency when in the anti-*his* orientation (Table 1). The differences in efficiencies between in vivo and in vitro are probably due to the secondary structures present in the *his* leader RNA that are known to affect the rate of termination (2). To overcome such effects, we linearized the DNAs by using restriction enzymes which have sites between the *his* terminator and the attenuator. pAR15 was cleaved with *Taq*I, and pAR1 and pAR16 were cleaved with *Hin*dIII. The transcripts synthesized in vitro with the linearized plasmid DNAs as templates are also shown in Fig. 7A. pAR1 (lane 4) still synthesized the 180-nt *his* leader transcript; pAR16 (lane 6) still produced the 185-nt RNA terminated at the *his* terminator, and pAR15 (lane 8) still produced the 280-nt RNA terminated at the *his* terminator in the opposite orientation. In both pAR15 and pAR16, the RNAs terminated at the *his* attenuator were no longer present. Moreover, if the termination process were not very efficient, one would expect to see in pAR16 cleaved with *Hin*dIII a 230-nt read-through transcript and in pAR15 cleaved with *Taq*I a 310-nt read-through transcript. Such transcripts were clearly not produced (Fig. 7A, lanes 6 and 8, respectively). We can therefore conclude that the *his* terminator functions very efficiently in both orientations not only in vivo but also in vitro.

DISCUSSION

In this paper, we provide evidence that a single Rho-independent site of transcription termination functions in both orientations to terminate RNA molecules which are convergently transcribed on opposite DNA strands.

The process of transcription termination is usually a polar phenomenon; that is, terminators only function when in proper orientation (15). On the other hand, by analyzing in more detail the sequences of many Rho-independent terminators, we have found that several of them possess a peculiar "mirror" structure. The G-C-rich stem is followed on both strands by a run of thymidylate residues (8, 16, 22, 23). This observation in turn suggests that such structures might function in both orientations to terminate convergent transcripts. In fact, such a role has been proposed for the T1 terminator of the *rrnD* operon of *E. coli* (8, 15).

The *S. typhimurium his* terminator possesses in high degree such organization. A perfect G-C stem of 6 bp with a $\Delta G(25°C)$ of -16.7 kCal (-69.9 kJ) (33) is flanked on both sides by a run of consecutive deoxyadenylate-deoxythymidylates 7 and 6 bp long, respectively (Fig. 1C). The structural characteristics of this site suggest that it could be used to terminate convergent transcripts. We have shown that this is indeed the case. The terminator is used in vivo to terminate in one direction the *his* operon mRNA, starting at both the *hisGp* primary promoter (10) and the internal promoters (14, 30). The in vivo S1 nuclease mapping experiments and the Northern analysis (Fig. 2 through 5) also demonstrate that the same site is used in the opposite direction to terminate a *his*-convergent RNA molecule 1,200 nt long of unknown function.

The efficiency of termination of transcription at this site in vivo in both orientations is extremely high, as shown by the reduction in expression of galactokinase activity in the pKL600 vector system (Table 1). The structure of the *his* terminator correlates very well with the characteristics of both synthetic and natural Rho-independent terminators required for maximum efficiency. The detailed analysis performed by Platt (24) showed in fact that the maximum useful length of the stem is 6 G-C bp followed by six or more consecutive T residues.

There is no easy way to ascertain whether the 3′ end of a transcript is the result of true termination at a site or is due to processing of longer molecules. In the case of the *his*

terminator, we cannot exclude a priori a processing event. The in vivo results with the pKL system indicate that processing sites, if present, should be contained in the cloned 110-bp fragment, that is, in the 43 bp beyond the run of T's in the *his* orientation and in the 34 bp in the opposite orientation.

The *his* terminator terminates transcription in both directions and very efficiently also in a purified in vitro system. The length of the transcripts is in perfect agreement with the expected size if the 3' ends coincide with the run of T's on both sides of the palindrome (Fig. 7).

Our data provide direct experimental evidence for the existence of overlapping terminators within the bacterial genome. The *his* terminator of *S. typhimurium* with its dual function could represent a very useful model system with which to study the process of transcription termination, especially since increasing evidence points to the importance of termination signals in the regulation of gene expression in procaryotes (15).

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