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Features of the Rho-Dependent Transcription Termination Polar Element within the *hisG* Cistron of *Salmonella typhimurium*

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Previous genetic analysis showed that the polar effects of mutations in the *hisG* cistron of *Salmonella typhimurium* are dependent on the presence of a single putative transcription termination element within the *hisG* gene. In fact, all proximal mutations causing translation termination are strongly polar, whereas distal ones are not. The element was mapped by isolating mutations able to relieve the polar phenotype, and they were found to be small deletions in the region downstream of the translational stop codon (M. S. Ciampi and J. R. Roth, Genetics 118:193–202, 1988). In this study, we analyzed the *his*-specific RNAs synthesized in vivo in different strains harboring the polar frameshift *hisG2148* mutation. The nature of the polarity effects is clearly transcriptional, since shorter RNA molecules were produced. When the *hisG2148* mutation was transferred in a *rho* background or in strains harboring the small distal deletions, an increase in readthrough transcription was observed. The transcriptional termination element was characterized in more detail by performing high-resolution S1 nuclease mapping experiments. This analysis showed that (i) termination or exonucleolytic degradation following termination produced transcripts with heterogeneous 3′ ends; (ii) this process is dependent on the transcription termination factor Rho, since relief of termination occurs in a *rho* background; and (iii) the element appears to function as a transcription terminator, at least to some extent, even in the course of active translation of the *hisG* cistron.

Several kinds of mutations in single cistrons within operons not only determine loss of the specific function encoded by the gene but can also cause polar effects. The reduction in the expression of the downstream cistrons is due to premature transcription termination mediated through the action of the Rho factor on defined regions of the nascent transcripts (1, 10). Despite many studies on the molecular events by which the Rho factor acts, the details of this mechanism are still poorly understood, since very few of these cryptic Rho-dependent elements unmasked by the uncoupling of transcription and translation within cistrons have been characterized (33, 35). In addition to Rho, many other cellular features participate in the transcription termination process: the RNA polymerase (35), a number of transcription factors (9, 14, 16, 19), and the overall structure and composition of defined regions of the mRNA molecules (33).

The *his* operon of *Salmonella typhimurium* has been extensively used as a model system to study polarity (27, 28), and a number of mutations, especially in the proximal cistrons (*hisG*, *hisD*, and *hisC*) (13, 29, 36), have been characterized at the genetic level. Genetic studies on a polar strain of *S. typhimurium* (*hisG2148*) have shown that the *hisG* gene contains a small region which is essential for polarity (6, 7). The region has been identified by isolating nonpolar revertants, which turned out to have small deletions in a limited region, confirming that defined sequences are required for or are involved in the process of transcription termination leading to polarity (6). We have recently shown that it is possible to perform transcriptional analysis of the events of transcription termination in polar mutants in vivo (2).

In the present study, we have performed a functional analysis of this region to obtain further information on the structures of these elements and on the mechanisms of the Rho-dependent transcription termination process. The RNAs produced by strains carrying the polar mutation *hisG2148*, the double mutation *hisG2148 rho-111*, and the deletion reversions *hisG2148 hitG199* and *hisG2148 hitG200* were isolated and analyzed by Northern (RNA) blot and S1 nuclease mapping. In the polar mutant, shorter *his* mRNA molecules were detected. In the presence of the *rho* mutation or either one of the two deletions, partial relief of the polar effects was observed. Discrete transcripts were mapped, in the region comprising the deletions, with 3′ ends coincident with five major sites possessing different structural features.

MATERIALS AND METHODS

Bacterial strains and plasmids. The following strains were used: *S. typhimurium hisO1242* (a 35-base-pair [bp] deletion of the attenuator region [25]), TR5998 (Δ*his-3050*), TR6215 (*metE338 hisO1242 hisC2124 trpE49 amt-49 ara-9 rho-111*) (6), SC685 (*hisO1242 hisG2148*), SC684 (*hisO1242 hisG2148 rho-111*), SC688 (*hisO1242 hisG2148 hitG199*), and SC689 (*hisO1242 hisG2148 hitG200*), and *E. coli* FB1 Δ(*hisGDC BHAFIE gnd*)*750 rhaA* (5). Strains SC684, SC685, SC688, and SC689 were isogenic, and they were obtained by first constructing two isogenic strains carrying a deletion of the entire *his* operon, *his-3050* (21), and either the *rho-111* mutation or the *rho*$^+$ allele. This was done by transferring the *rho-111* mutation by transduction in a *his-3050* deletion strain. This was made possible by the availability of a Tn*10* insertion in the *ilv* operon, which is located near *rho*. The tetracycline resistance associated with Tn*10* was used as a selective marker to transfer the *rho-111* mutation into the *his-3050* deletion strain. The Tn*10* element was then re-

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moved by transducing the resulting strain, *his-3050 ilv*::Tn*10 rho-111*, to *ilv*$^+$, and a *rho*$^+$ and a *rho* recombinant were saved from this cross. The *his* operon region of strains carrying the *hisG2148* polar mutation or *hisG2148* in combination with either the *hitG199* or the *hitG200* terminator mutation was then transferred in the *his-3050 rho*$^+$ strain to obtain SC685, SC688, and SC689, respectively. The isogenic strain SC684 was obtained by transferring the *his* operon region of *hisG2148* in the *his-3050 rho-111* strain. Transductional crosses were performed by the method of Johnston and Roth (24), and in all transductions the mutant P22 phage HT105/1 *int201* was used (39). This phage transduces at high frequency and carries an *int* mutation isolated by G. Roberts. Phage was grown by the method of Hoppe et al. (22). When selecting for tetracycline resistance, bacterial cells and phages were mixed and incubated for 20 min at 37°C before being plated on selective medium. The expression vector pEMBL8, containing the *lac* control region (11), was used to clone a genomic *Bgl*II-*Hin*dIII fragment of 2,980 bp (Fig. 1) containing the proximal *S. typhimurium his* operon region in the *Bam*HI and *Hin*dIII sites of the polylinker. The genomic DNA of strain SC685 was isolated and restricted as previously described (5). Recombinant plasmid pHS2148 was isolated by double selection for antibiotic resistance and growth on histidinol (selecting for HisD$^+$) of the *E. coli* auxotrophic strain FB1. Plasmid DNA was purified by the method of Clewell (8).

Growth conditions. The strains were grown in minimal medium (43) containing 0.5% glucose, and when required ampicillin (50 μg/ml), tetracycline (10 μg/ml in minimal medium or 15 μg/ml in rich medium), and 10 mM histidinol were added. Rich medium was Luria-Bertani broth (32). Strains harboring the temperature-sensitive *rho-111* mutation (23) were grown either at the permissive temperature (30°C) or at 37°C.

Purification, labeling, and sequencing of DNA fragments. DNA fragments or strands were isolated through acrylamide slab gels and recovered by electroelution as described previously (26). Single-stranded DNA from M13 clones was prepared as described by Messing et al. (31). 3′-End labeling was performed with reverse transcriptase (18) or with the Klenow fragment of DNA polymerase (3). Nick translation was performed by the method of Rigby et al. (37). DNA sequencing was performed by the dideoxy-chain termination procedure (38). The sequence of the region spanning the *hisG2148* mutation was obtained by subcloning an *Eco*RI-*Hin*cII fragment in the phage vectors M13mp18 and M13mp19 (45). For the high-resolution S1 nuclease mapping experiments, the sequencing lanes were obtained by the Maxam and Gilbert (30) sequencing technique. DNA sequence analysis and a search for RNA secondary structures were performed by using the Microgenie program (Beckman Instruments, Inc.) and an IBM computer.

RNA preparation, Northern blot analysis, and S1 mapping. Total bacterial RNA was extracted from logarithmically growing cells by the guanidine hydrochloride procedure described previously (20). Electrophoretic analysis was done by fractionating the total RNA on 1% agarose gels containing formaldehyde (17; as modified by Maniatis et al. [26]). RNA transfer to Hybond (Amersham Corp.) membranes and hybridization with ^{32}P-labeled fragments were performed by the method of Thomas (41). RNA-DNA hybridization, S1 nuclease digestion, and analysis of the hybrids on polyacrylamide denaturing gels were performed as described elsewhere (12). The reaction mixtures contained different amounts of labeled DNA probes and different amounts of specific RNA (see the legends to Fig. 4 and 5). The autoradiograms were scanned by densitometry with an LKB 2202 UltroScan Laser Densitometer to obtain quantitative data. Further analysis was performed by cutting radioactive bands from the gel and counting the strips of acrylamide thus obtained in 5 ml of HP (Beckman). Values were expressed as percentages of the values obtained for the controls.

RESULTS

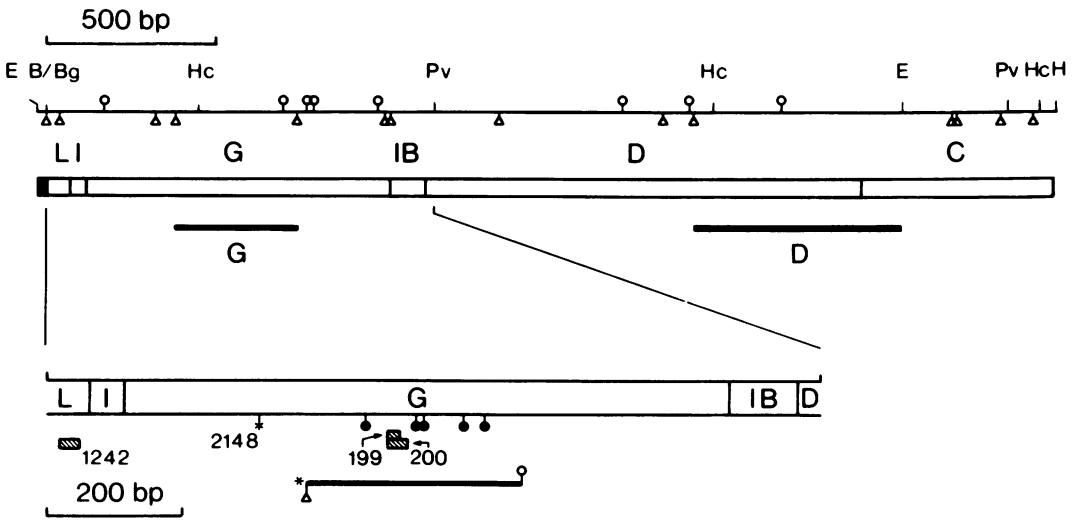
Cloning and sequencing of the *hisG2148* mutation. Figure 1A shows a restriction map of the proximal region of the *S. typhimurium his* operon. The *Bgl*II-*Hin*dIII genomic fragment was cloned from the polar mutant strain SC685 (*hisO1242 hisG2148*). This DNA fragment is 2,980 nucleotides long and contains the *hisG*, *hisD*, and part of the *hisC* cistrons (4). The fragment was cloned in the vector plasmid pEMBL8 (11). The features of this region and the positions of the relevant genetic elements are presented in Fig. 1A. To define the DNA changes that caused the *hisG2148* mutation, we determined the nucleotide sequence of the 470-bp *Eco*RI-*Hin*cII fragment spanning the proximal region of the *hisG* cistron. The mutation is a deletion of one G residue at nucleotide 199 of the wild-type *hisG* cistron (5). The effects of this genotypic change on the translation product are shown in Fig. 1B. The *hisG2148* mutation causes the loss of the normal translational frame after the amino acid residue at position 66 and the synthesis of an out-of-frame peptide of 27 amino acids until a new stop codon (TAA) is encountered after 280 nucleotides from the beginning of the *hisG* cistron. This change results in the synthesis of a truncated *hisG* polypeptide of 93 amino acids.

Northern blot analysis of the *his* mRNAs. We next determined the transcriptional pattern of the *hisG2148* mutation. Total RNA was extracted from the parental (*hisO1242*) and mutant (SC685) strains and analyzed by Northern blots using different *his*-specific genomic fragments (Fig. 2) as probes. A proximal *hisG*-specific DNA probe hybridized in the parental strain to long heterogeneous transcripts, including full-length *his* mRNA molecules of 7,390 nucleotides transcribed from the P1 promoter (5) (Fig. 2, lane 1), whereas in the polar mutant strain SC685 a much shorter RNA species of about 700 nucleotides was detected (Fig. 2, lane 2). A distal *hisD*-specific probe hybridized to the same long mRNA molecules in the parental strain (Fig. 2, lane 3), whereas no mRNA was detected in strain SC685 (Fig. 2, lane 4). These data indicate that in the mutant strain shorter *his* mRNA molecules were produced and that the events by which they are generated must be very efficient.

To determine the effects of the termination factor Rho and of the small deletions causing relief of polarity, we performed Northern blots with the RNAs extracted from strains SC685 (*hisO1242 hisG2148*), SC684 (*hisO1242 hisG2148 rho-111*), SC688 (*hisO1242 hisG2148 hitG199*), and SC689 (*hisO1242 hisG2148 hitG200*) (Fig. 3). When the RNAs were hybridized to the proximal probe, the 700-nucleotide RNA was again detected in the SC685 strain (Fig. 3, lane 1), whereas longer RNA molecules were produced in strain SC684 grown at 37°C (Fig. 3, lane 2) and in strains SC688 and SC689 (Fig. 3, lanes 3 and 4). In addition, when the same RNAs were hybridized to a distal probe no detectable RNA was present in strain SC685 (Fig. 3, lane 5), and the same longer RNA molecules were produced in strains SC684, SC688, and SC689 (Fig. 3, lanes 6 to 8).

The Northern blot experiments with distal probes failed to reveal readthrough transcripts which must be present in the

A



B

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        10         20         30         40         50         60         70         80
ATGTTAGACAACACCCGCTTACGCATAGCTATTCAGAAATCAGGCCGTTTAAGCGATGATTCACGAGAATTGCTGGCCCG
MetLeuAspAsnThrArgLeuArgIleAlaIleGlnLysSerGlyArgLeuSerAspAspSerArgGluLeuLeuAlaAr

        90        100        110        120        130        140        150        160
CTGCGGCATAAAAATTAATTTACACACTCAGCGCCTGATTGCGATGGCGGAAAACATGCCGATTGATATCCTGCGCGTGC
gCysGlyIleLysIleAsnLeuHisThrGlnArgLeuIleAlaMetAlaGluAsnMetProIleAspIleLeuArgValA

       170        180        190        200        210        220        230        240
GTGATGATGACATTCCGGGTCTGGTAATGGATGGCGTGgTCGATCTCGGTATTATCGGCGAAAACGTGCTGGAAGAAGAG
rgAspAspAspIleProGlyLeuValMetAspGlyVal SerIleSerValLeuSerAlaLysThrCysTrpLysLysSe

       250        260        270        280        290        300        310        320
CTACTCAACCGCCGCGCACAGGGCGAAGATCCACGCTATTTAACCCTGCGCCGTCTTGACTTCGGCGGCTGCCGTTTATC
rTyrSerThrAlaAlaHisArgAlaLysIleHisAlaIle***

       330        340        350        360        370        380        390        400
GCTGGCAACACCGGTTGACGAAGCCTGGGACGGCCCGGCCGCGCTGGACGGTAAACGTATCGCTACCTCATATCCGCACC

       410        420        430        440        450        460                   480
TCCTCAAACGCTACCTCGACCAGAAAGGCGTCTCTTTTAAATCGTGTCTGTTAAATGGTTCTGTCGAAGTCGCGCCGCGC
          199        200

       490        500        510                   530        540        550        560
GCGGGGCTGGCCGACGCTATCTGCGATTTGGTCTCTACCGGCGCGACGCTTGAAGCTAACGGCCTGCGTGAAGTCGAAGT

       570        580        590        600        610        620        630        640
TATCTACCGCTCTAAAGCCTGTCTGATTCAGCGCGACGGTGAGATGGCACAGAGCAAGCAAGAGCTGATCGATAAATTGC

       650        660        670        680        690        700        710        720
TGACCCGTATTCAGGGCGTGATTCAGGCGCGCGAATCGAAATACATCATGATGCACGCGCCAAGTGAACGCCTGGAAGAG

       730        740        750        760        770        780        790        800
GTTATCGCCCTGCTGCCAGGCGCCGAAAGGCCGACAATTCTGCCGCTGGCAGGCGAGCAACAGCGCGTGGCGATGCACAT

       810        820        830        840        850        860        870        880
GGTCAGCAGCGAAACGTTGTTCTGGGAAACCATGGAGAAACTGAAAGCGCTTGGCGCCAGCTCGATTCTGGTACTGCCGA

       890        900
TCGAGAAGATGATGGAGTGA
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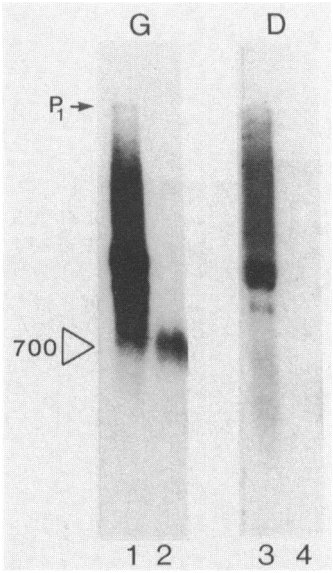

FIG. 2. Northern blot analysis of the *his* RNAs transcribed in vivo. Total RNA (10 μg) extracted from the parental (*hisO1242*, lanes 1 and 3) and mutant (SC685, lanes 2 and 4) strains was electrophoresed on 1% agarose–formaldehyde gels, transferred to Hybond-N (Amersham) membranes, and hybridized to nick-translated DNA probes corresponding to different regions of the operon. The probes used in the hybridization experiments (G, lanes 1 and 2, or D, lanes 3 and 4) are shown in Fig. 1A. Positions of the full-length message transcribed from the primary (P_1) promoter and the shorter heterogeneous transcripts (700) produced in strain SC685 are indicated.

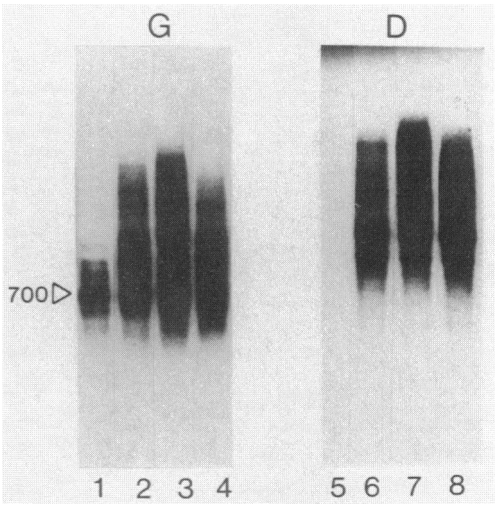

FIG. 3. Relief of transcription termination in the presence of *rho-111* and *hitG199* and *hitG200* mutations. Total RNA (10 μg) extracted from the mutant strains SC685 (lanes 1 and 5), SC684 grown at 37°C (lanes 2 and 6), SC688 (lanes 3 and 7), and SC689 (lanes 4 and 8) was treated, and the filters were hybridized with the two probes (G, lanes 1 to 4, and D, lanes 5 to 8) as described in the legend to Fig. 2. The position of the shorter transcripts (700) produced in strain SC685 is shown.

hisG2148 mutant, since the strain expresses the enzymes coded for by the distal cistrons (6). To obtain a measure of the reduction in the amount of readthrough transcription, we performed a quantitative S1 nuclease protection experiment (Fig. 4) with total RNA extracted from the *hisO1242* and SC685 strains and hybridized to a *Bgl*II-*Pvu*II 1,145-bp probe (Fig. 1A) labeled at the 3′ end. The amount of distal *his*-specific RNA present in the strains was established by densitometry and scintillation counting (see Materials and Methods) of the full-length-protected hybrids. The RNA levels detected in the mutant strain SC685 (Fig. 4, lane 2) were 10% of those present in the parental strain (*hisO1242*) (Fig. 4, lane 1). In addition, the same analysis allowed us to determine more precisely the effect induced by the *rho* mutation on the relief of polarity. In strain SC684 the readthrough was increased to 50% (Fig. 4, lane 3). These data demonstrate that the polarity of the mutant is due to premature Rho-dependent transcription termination.

Mapping of the 3′ ends of the transcripts. We analyzed the region where the Rho factor acts in vivo by S1 nuclease protection experiments (Fig. 5). The total RNAs extracted from the different strains were hybridized to the 320-bp *Sau*3AI-*Hin*fI fragment (Fig. 1A and Fig. 5, lane 2) labeled at the 3′ end of the coding strand. The analysis by high-resolution S1 mapping showed that multiple shorter RNAs with heterogeneous 3′ ends are present in the mutant strain SC685 (Fig. 5, lane 3). Five major endpoints were identified, at 90, 167, 177, 234, and 265 nucleotides from the labeled 3′ end of the probe. They account for 80% of all the endpoints present in the mutant, as measured by scanning of the autoradiogram. A reduced amount of these transcripts was present in strain SC684 carrying the *rho* mutation (Fig. 5, lane 4), and very low amounts of the second, fourth, and fifth transcripts were observed even in the parental strain (Fig. 5, lane 5). The nucleotide sequence and the most prominent

FIG. 1. Structural features and genetic map of the proximal region of the *S. typhimurium his* operon. (A) Physical and genetic maps of the chromosomal region spanning the *hisG* cistron. Top line: restriction map of the 2,980-bp *Bgl*II-*Hin*dIII fragment (5) isolated from strain SC685 and cloned in vector plasmid pEMBL8 (11). Restriction sites: E, *Eco*RI; B/Bg, border between the *Bam*HI site of the vector and the *Bgl*II site in the chromosomal DNA; Hc, *Hin*cII; Pv, *Pvu*II; H, *Hin*dIII; ○, *Hin*fI sites; △, *Sau*3AI sites. Middle line: Relative positions and lengths of the genetic elements contained in the fragment. L, *his* leader; I, intercistronic region between *hisL* and *hisG* (G) cistrons; IB, intercistronic barrier between the *hisG* and the *hisD* (D) cistrons; C, *hisC* cistron. ■, Polylinker sequences of the vector. Relative positions of the 370-bp *Sau*3AI (G) and the 620-bp *Sau*3AI-*Eco*RI (D) fragments used as proximal and distal probes, respectively, in the Northern blot experiments are indicated. Bottom line: enlarged diagram of the 1,145-bp *Bgl*II-*Pvu*II fragment spanning the *hisG* cistron used in this study for the quantitative S1 protection experiments. Genetic elements are indicated as described above. The relevant features of this region are shown below the map. ▨, Deleted regions (1242, 35-bp deletion of the *his* attenuator [25]; 199 and 200, deletions relieving the polar phenotype of the *hisG2148* [2148*] mutation [6]). ●, 3′ ends of the five major transcripts identified in the S1 nuclease mapping experiments using the 320-bp *Sau*3AI-*Hin*fI fragment (——) labeled (*) at the 3′ end of the coding strand. (B) Nucleotide sequence of the *hisG2148* cistron. Nucleotides are numbered from the first base of the *hisG* gene. The deleted G nucleotide (199) is indicated by a lowercase character. The *nusA*-like site (360 to 367) is boxed. Sequences deleted in *hitG199* (390 to 407) and *hitG200* (390 to 417) are underlined. Vertical arrows identify the 3′ ends of the transcription termination events. Sequences capable of forming secondary structures are shown by converging arrows below (343 to 349:354 to 359; 469 to 478:486 to 495) or above (469 to 477:519 to 527) the sequence. The amino acid sequence of the altered gene product is indicated below the nucleotide sequence, and the out-of-frame carboxyl-terminal end is underlined. ***, TAA stop codon.

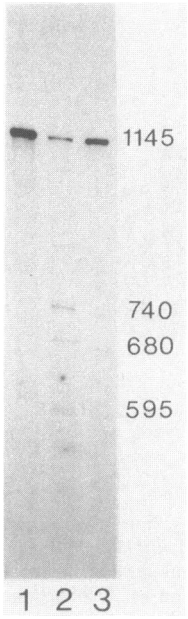

FIG. 4. Quantitative S1 nuclease mapping of *his*-specific transcripts in the different strains. Fifty nanograms of the 3'-labeled 1,145-bp *Bgl*II-*Pvu*II DNA fragment (Fig. 1A) was hybridized with 50 μg of total RNA extracted from strain *hisO1242* (lane 1), SC685 (lane 2), or SC684 grown at 37°C (lane 3) and treated with S1 nuclease. The quantitative analysis was performed as described in Materials and Methods. A labeled *Sau*3AI digest of the 1,145-bp *Bgl*II-*Pvu*II DNA fragment was run as a reference marker (not shown) and was used to calculate the sizes of the different protected transcripts, which are indicated (in base pairs) on the right.

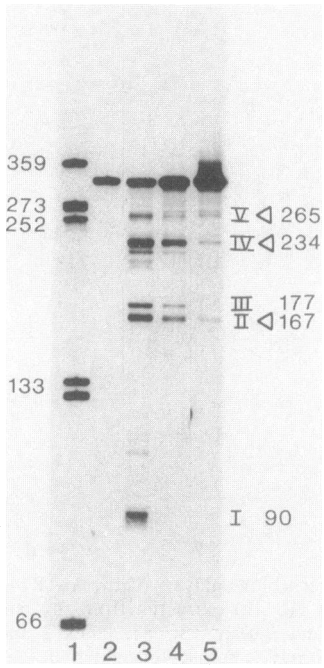

FIG. 5. High-resolution S1 nuclease mapping of the 3' termini of RNAs. The *Sau*3AI-*Hin*fI 320-bp fragment corresponding to nucleotides 270 to 590 of the *hisG* cistron was labeled at the 3' end (lane 2) of the coding strain and hybridized with 50 μg of total RNA extracted from strains SC685 (lane 3), SC684 grown at 37°C (lane 4), and *hisO1242* (lane 5). Hybrids were treated with S1 nuclease and analyzed on a 6% acrylamide–8 M urea sequencing gel, in parallel with purine reaction sequencing ladders of the same fragment (not shown) obtained with the Maxam and Gilbert technique. The positions of the five major stop sites (I, II, III, IV, and V) and the number (in nucleotides) from the labeled 3' end of the probe are marked on the right. Arrowheads indicate the three shorter transcripts that are also present in the *hisO1242* strain. In lane 1, a labeled *Sau*3AI digest of the 1,145-bp *Bgl*II-*Pvu*II DNA fragment is shown as a reference marker, and the sizes (in base pairs) of the different fragments are indicated on the left.

structural features of this region, as well as the relative positions of the small deletions and the nucleotides corresponding to the different 3' ends of the transcripts, are presented in Fig. 1B.

DISCUSSION

We have chosen polar mutations in the *his* operon of *S. typhimurium* as a model system to study the Rho-dependent transcription termination process (2, 6). Polarity depends on the existence of a defined region(s) on the nascent transcripts that could represent the genetic signal(s) for Rho action and which is located between the nonsense codon and the next reinitiation of translation site (1, 34).

The *hisG2148* frameshift mutation determines a translational stop very early in the coding region (Fig. 1). As a consequence of this event, the nascent RNA molecules are no longer protected by the ribosomes and the latent transcription termination element is activated. In fact, the analysis of the RNA produced in vivo in the mutant strain showed that shorter transcripts were generated (Fig. 2) and that extremely low amounts of distal sequences were transcribed, indicating that the termination event was very efficient. Previous biochemical evidence has shown that expression of downstream genes is only 5% of wild-type levels (13). The quantitative analysis of readthrough transcription (Fig. 4) is in agreement with these estimates and indicates that the reduced expression of the distal cistrons is entirely transcriptional. It was previously shown that in a *rho* genetic background and in strains harboring small deletions, which were mapped downstream of the mutation, there was a relief of the polar phenotype (6). These effects too are transcriptional, since in each case the termination element was no longer functional and readthrough transcripts were produced (Fig. 3).

A molecular analysis in vivo of the polar effects of nonsense mutations, in combination with formal genetic experiments, constitutes a useful model system in which to study relevant physiological phenomena of the process of transcription termination. One limitation of the methods used in the present work is that the experiments in vivo detect steady-state RNAs and therefore do not help to discriminate whether the observed 3' ends of the transcripts are coincident with real termination sites or are the result of processing events. Only a combination of in vivo experiments and in vitro studies using purified factors will be able to resolve this issue.

The high-resolution S1 mapping analysis showed that the termination process resulted in the production of heterogeneous transcripts with different 3' ends (Fig. 1 and 5). The five major sites coincident with transcription endpoints have the following features. (i) The first one occurs immediately downstream of a region possessing the potential to form a secondary structure with a ΔG^0 of -12 kcal (42) within a sequence that resembles the consensus element (*boxA*) for the NusA factor (15). The second and third ones are close to

each other (10 nucleotides apart) and are within a region devoid of secondary structures. The fourth and fifth ones are again found immediately downstream of sequences capable of forming secondary structures with ΔG^0s of −21 and −23 kcal, respectively, and the entire region from nucleotides 468 to 533 has the potential to form several secondary structures.

The function of the cryptic transcription termination element unmasked by the *hisG2148* polar mutation is controlled by the Rho factor. In fact, in the *rho* background, readthrough transcription was restored to 50% of the wild-type levels (Fig. 4). In addition, the high-resolution S1 protection experiment showed that Rho affects each of the five major discrete transcripts, since the mutated Rho protein caused a decrease in their relative amounts. The extent of Rho action was not constant: in fact, the first was the most affected and the fourth one was the least affected. The other three exhibited intermediate decreases (III > V > II) (Fig. 5). This probably depends on the different structural features of the sites, which in turn can cause either variable efficiency of Rho-RNA polymerase interaction if they are real terminators or variable protection to exonucleases if they are processing sites.

The exposed region of the nascent mRNA molecules from the nonsense codon to the deleted sequences in *hit-199* and *hit-200* is 140 nucleotides long (from nucleotide 280 to nucleotide 420). However, other mutations that map more distally than *hisG2148* still have a polar phenotype (6; unpublished data). The small deletions that cause relief of the polar phenotype by suppressing transcription termination map distal to the first and proximal to the last four transcription endpoints. The deleted sequences therefore do not act by eliminating termination sites but must contain elements that are essential for the occurrence of the transcription termination process.

Another interesting observation is that the second, fourth, and fifth discrete transcripts are also present, although in small amounts, in the wild-type strain and in the presence of active translation. This result is in keeping with the evidence provided by us for the *hisC* cistron (2) and that obtained for the *lacZ* cistron by Stanssens et al. (40). It is possible that such sites might correspond to polymerase pause sites such as those described in vitro by several authors (for a review, see reference 44).

This transcription termination element and the one unmasked by early polar mutants within the *hisC* cistron, which we have recently described (2), have many similar characteristics. Common features are the presence of consensus sequences for Nus factors near some 3′ ends, potential for some regions of the RNA to form secondary structures, heterogeneous endpoints, dependence on Rho factor, and quantitative differences in the extent of Rho effects. The analysis of many of these elements currently in progress in our laboratories will help clarify the features responsible for the Rho-dependent transcription termination process.

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