Enhancing Transcription through the *Escherichia coli* Hemolysin Operon, *hlyCABD*: RfaH and Upstream JUMPStart DNA Sequences Function Together via a Postinitiation Mechanism

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*Escherichia coli* hlyCABD operons encode the polypeptide component (HlyA) of an extracellular cytolytic toxin as well as proteins required for its acylation (HlyC) and sec-independent secretion (HlyBD). The *E. coli* protein RfaH is required for wild-type hemolysin expression at the level of *hlyCABD* transcript elongation (J. A. Leeds and R. A. Welch, J. Bacteriol. 178:1850–1857, 1996). RfaH is also required for the transcription of wild-type levels of mRNA from promoter-distal genes in the rfaQ-K, *traY-Z*, and *rplK-rpoC* gene clusters, supporting the role for RfaH in transcriptional elongation. All or portions of a common 39-bp sequence termed JUMPStart are present in the untranscribed regions of RfaH-enhanced operons. In this study, we tested the model that the JUMPStart sequence and RfaH are part of the same functional pathway. We examined the effect of JUMPStart deletion mutations within the untranscribed leader of a chromosomally derived *hlyCABD* operon on hly RNA and HlyA protein levels in either wild-type or *rfaH* null mutant *E. coli*. We also provide in vivo physical evidence that is consistent with RNA polymerase pausing at the wild-type JUMPStart sequences.

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TABLE 1. Recombinant plasmids and E. coli strains used in this study

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<tr>
<td>pSF4000</td>
<td>936 hlyCABD operon in PACYC184</td>
<td>This laboratory (33)</td>
</tr>
<tr>
<td>pWAM2240</td>
<td>pSF4000 (Δ+126+137)</td>
<td>This study</td>
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<tr>
<td>pWAM2245</td>
<td>pSF4000 (Δ+149+159)</td>
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<td>pWAM744</td>
<td>1,386-bp pSF4000 HindIII-EcoRI fragment; contains hlyBD in opposite orientation to SP6 promoter in pGEMIE; source of hlyBD 149 to +6410 probe</td>
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<td>pWAM1452</td>
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<td>pWAM2098</td>
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E. coli strains

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RESULTS

Deletion of two highly conserved regions of the JUMPStart sequence from a chromosomally derived hlyCABD operon. We and others have shown that the E. coli protein RfaH enhances hlyCABD mRNA expression both in vivo (20) and in vitro (1). Results from the in vivo study demonstrated that RfaH is not required for initiation of hlyCABD transcription from the native hly promoter or for stability of hly mRNA (20). The in vitro study showed that transcription of the hlyCABD genes under the control of a heterologous promoter is increased in the presence of purified RfaH (1). These data are consistent with a role for RfaH in enhanced elongation of transcription. RfaH also increases steady-state mRNA levels from several other gene clusters in E. coli and S. typhimurium (4, 5, 26–28).

JUMPStart is a conserved sequence within the untranslated regions of all RfaH-affected operons identified to date (15, 29). The function of the JUMPStart sequence is unclear. In this study, we tested the hypothesis that the JUMPStart sequence and RfaH function in the same pathway. We characterized two deletion mutations of the 5' noncoding region of a hlyCABD operon derived from uropathogenic E. coli 396 in wild-type and null rfaH mutant backgrounds. The deletions were derived by PCR-based mutagenesis of the hly subclone pWAM2098. The mutagenized DNA was substituted for the wild-type sequence in the intact hlyCABD operon on pSF4000 (Table 1). Figure 1 depicts the alignment of the hlyCABD sequence in pSF4000 with the JUMPStart consensus sequence defined by Hobbs and Reeves (15). In the alignment of all known JUMPStart sequences, gaps frequently occur within the upstream subsequence and within the spacer between the two subsequences. No gaps have been identified within the downstream JUMPStart subsequence (18). The deletion in pWAM2240 removes the region from positions +126 to +137, including most of the first, moderately conserved JUMPStart subsequence. The deletion in pWAM2245 removes the region from positions +149 to +159, including the absolutely conserved 5' GGCGGTAG.
rfaH null allele significantly reduces the hemolytic-zone size (2, 19). When transformed into the wild-type E. coli RZ4500, both JUMPStart deletions conferred reduced zones of hemolysis compared to those produced by colonies transformed with the intact pSF4000 plasmid (19). Transformation of the JUMP-Start deletions into the rfaH null mutant E. coli WAM1925 resulted in zone sizes similar to that conferred by the wild-type pSF4000 in the rfaH null mutant background. The observation that the effect of the JUMPStart deletions on zone size was not compounded by the rfaH null allele suggested that one is epistatic to the other or that RfaH and JUMPStart are part of the same functional pathway.

Effect of JUMPStart deletions on HlyA protein levels in wild-type and rfaH null mutant E. coli. To determine the effect of the JUMPStart deletions on extracellular HlyA expression, we compared the levels of extracellular HlyA precipitated from wild-type or rfaH null mutant E. coli culture filtrates by SDS-polyacrylamide gel electrophoresis and staining with Coomassie brilliant blue. Deletion of the JUMPStart sequences resulted in 8- and 16-fold reductions in extracellular HlyA from E. coli harboring pSF4000 plasmid (19). Transformation of the JUMP-Start deletions into the rfaH null mutant E. coli WAM1925 also resulted in a significant reduction in extracellular HlyA compared to that from the wild-type pSF4000 in the rfaH null mutant background. The observation that the effect of the JUMPStart deletions on zone size was not compounded by the rfaH null allele suggested that one is epistatic to the other or that RfaH and JUMPStart are part of the same functional pathway.

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Results: Effect of JUMPStart deletions on HlyA mRNA levels. We previously demonstrated that the reduction in HlyA protein levels from rfaH null mutant E. coli correlates with decreased steady-state levels of hlyCABD mRNA (20). To see if the JUMPStart sequences, like RfaH, affect hlyCABD mRNA levels, we performed quantitative RNAse protection analyses on total RNAs extracted from the wild-type and rfaH null mutant E. coli harboring pSF4000 or one of the two deletions. The antisense RNA probes used are shown in Fig. 3A, and the results of RNase protection assays are shown in Fig. 3B and C. The multiple +779 to +1049 probe signals (hlyCa) reflect the two RNA species that result from in vitro transcription of the template DNA (1, 18). Probes protected by hlyC mRNA also migrate faster than the control probes. The incomplete protection is due to in vivo processing of hlyC mRNA (20).

Deletion of either JUMPStart sequence resulted in a significant reduction in hlyCABD mRNA from wild-type E. coli (Fig. 3B and C; rfaH+ lanes; compare wild-type signals with signals from JUMPStart mutants [note that the mutants shown in Fig. 3B and C were cloned into the pWAM2245 plasmid]) and eliminated hlyA mRNA from the JUMPStart-deleted null mutant signals (lanes 3 to 5). When examined in an rfaH null mutant background, the JUMPStart deletions did not compound the effect of the rfaH mutation on extracellular HlyA levels (Fig. 2B, compare rfaH null mutant signals [lanes 3 to 5] with signals from JUMPStart deletion mutants in the rfaH null mutant background [lanes 6 to 9]). Therefore, the reductions in HlyA levels conferred by the JUMPStart deletions in rfaH+ E. coli are similar to the reduction conferred by the null rfaH mutation on HlyA expression from either the intact pSF4000 (20) or JUMPStart deletion mutants.

JUMPStart deletions, like the rfaH null mutation, reduce steady-state hlyCABD mRNA levels. We previously demonstrated that the reduction in HlyA protein levels from rfaH null mutant E. coli correlates with decreased steady-state levels of hlyCABD mRNA (20). To see if the JUMPStart sequences, like RfaH, affect hlyCABD mRNA levels, we performed quantitative RNAse protection analyses on total RNAs extracted from the wild-type and rfaH null mutant E. coli harboring either wild-type or JUMPStart-deleted hlyCABD operon on pSF4000, which extends from the start of transcription (+1) to the start of translation of hlyC (+485). The bottom line of sequence depicts the JUMPStart consensus (underlined) and extended sequence as defined by Hobbs and Reeves (M, A or C; S, G or C; R, G or A; Y, C or T; W, T or A; N, A or C or G or T) (15). Gaps (–) were inserted to optimize the alignment. Vertical lines indicate sequence matches. Boldface indicates JUMPStart consensus subsequences as defined by sequence alignments (18). Δ+126–137, bases deleted in pWAM2240; Δ+149–159, bases deleted in pWAM2245. The asterisk denotes a T conserved among all JUMPStart sequences identified to date.

FIG. 1. Alignment of the hlyCABD leader region with the JUMPStart consensus sequence and locations of hlyCABD JUMPStart deletions. The top line of sequence depicts a portion (+119 to +170) of the untranslated leader region of the hlyCABD operon on pSF4000, which extends from the start of transcription (+1) to the start of translation of hlyC (+485). The bottom line of sequence depicts the JUMPStart consensus (underlined) and extended sequence as defined by Hobbs and Reeves (M, A or C; S, G or C; R, G or A; Y, C or T; W, T or A; N, A or C or G or T) (15). Gaps (–) were inserted to optimize the alignment. Vertical lines indicate sequence matches. Boldface indicates JUMPStart consensus subsequences as defined by sequence alignments (18). Δ+126–137, bases deleted in pWAM2240; Δ+149–159, bases deleted in pWAM2245. The asterisk denotes a T conserved among all JUMPStart sequences identified to date.
show that, similar to the effect on downstream 
*hlyCABD* operon. The level of *hlyA* message was reduced 6- and 7-fold, and the level of *hlyBD* message was reduced 14- and 20-fold, from the same constructs. The *rfaH* null allele alone reduced the 3′ end of the *hlyC* message 9-fold, *hlyA* mRNA 6-fold, and *hlyBD* mRNA 9-fold from the intact *hlyCABD* operon, compared to the wild-type strain (Fig. 3B and C, compare signals from pSF4000 in *rfaH*° versus *rfaH* null mutant *E. coli*). When examined in an *rfaH* null mutant background, the JUMPStart deletions reduced the 3′ end of the *hlyC* message 9- and 11-fold, *hlyA* mRNA 8- and 10-fold, and *hlyBD* mRNA 10- and 14-fold (Fig. 3A and B, compare pSF4000 signals in *rfaH*° *E. coli* with signals from JUMPStart mutants of *rfaH* mutant *E. coli*). These data indicate that expression of the JUMPStart deletion constructs in the *rfaH* null mutant strain of *E. coli* did not compound the effect on *hlyCABD* mRNA levels of either the JUMPStart deletions or the null *rfaH* allele alone. These data are consistent with the model that RfaH and JUMPStart are part of the same functional pathway.

**Effect of the JUMPStart deletions and the *rfaH* null allele on steady-state levels of RNA from the *hly* leader and 5′ end of *hlyC*.** Previous work showed that the *rfaH* null allele does not reduce leader and 5′ *hlyC* mRNA expression from pSF4000 to the extent that it reduces levels of downstream *hlyCABD* mRNA (18, 20). We performed RNase protection analyses to compare levels of *hly* leader and 5′ *hlyC* mRNA from the intact and JUMPStart-deleted *hlyCABD* operons in wild-type and *rfaH* null mutant *E. coli*. Figure 4A shows the location of an antisense probe to a region within the *hlyCABD* leader, extending into *hlyC*. The data in Fig. 4B depict the profile of the antisense probe following an RNase protection assay, as described in the legend to Fig. 3.

Deletion of the JUMPStart sequences resulted in two- and threefold reductions in *hly* leader-5′ *hlyC* mRNA from wild-type *E. coli* (Fig. 4B and C; in *rfaH*° lanes, compare wild-type signals with signals from JUMPStart mutants [note that equal amounts of total RNA were assayed for each strain]). When examined in an *rfaH* null mutant background, the JUMPStart deletions expressed amounts of *hly* leader-5′ *hlyC* mRNA similar to those expressed from those plasmids in wild-type *E. coli* (Fig. 4B and C; compare JUMPStart signals in *rfaH*° lanes and in *rfaH* mutant lanes). The *rfaH* null allele alone conferred less than a twofold reduction in *hly* leader-5′ *hlyC* mRNA levels (Fig. 4B and C; compare pSF4000 signals in *rfaH*° lanes with pSF4000 signals in *rfaH* mutant lanes).

The data in Fig. 4 demonstrate that expression of the JUMPStart deletions in either wild-type or *rfaH* null mutant *E. coli* reduced *hly* leader and 5′ *hlyC* mRNA to a lesser degree than they reduced downstream *hlyCABD* mRNA. The data also show that, similar to the effect on downstream *hlyCABD* mRNA, JUMPStart deletions in combination with the *rfaH* null allele did not result in a compounded reduction in *hly* mRNA expression beyond the effect of either mutation by itself. The 2- to 3-fold reduction in 5′ *hly* RNA conferred by the null *rfaH* allele or the JUMPStart deletions may have been caused by premature transcript termination or increased mRNA decay in the *hly* leader region. The experiments presented in this work do not address the effect of the JUMPStart sequences on *hlyCABD* message decay, and we therefore cannot rule out a role for the JUMPStart sequences in downstream mRNA stability. However, our previous work demonstrated that RfaH is not required for *hly* mRNA stability (20). Although the precise nature of the JUMPStart effect remains to be determined, the two- to threefold reduction in upstream *hly* mRNA did not fully account for the reduction in extracellular *Hly* levels from these strains. The RNase protection data presented in Fig. 3 and 4 favor the hypothesis that RfaH and JUMPStart are part of the same functional pathway, and these data are consistent with a model for the JUMPStart sequences in an RfaH-dependent elongation of *hly* transcription.

RNA polymerase may pause at the JUMPStart sequence. We and others have proposed a model for RfaH-enhanced gene expression at the level of transcription elongation (1, 4, 9, 13, 20). This work demonstrates that removal of either RfaH, JUMPStart sequences, or both leads to similar reductions in steady-state *hlyCABD* transcript levels in vivo, suggesting that they are part of the same functional mechanism. Recent in vitro experiments support this hypothesis, since removal of RfaH or the JUMPStart sequences reduces transcription of a hybrid construct containing the *hlyCABD* genes expressed from the *tac* promoter (1).

If transcriptional elongation of *hlyCABD* mRNA depends on alteration of the transcription machinery, including, minimally, RNAP, then pausing of RNAP during transcription may allow the necessary cofactors (cis and/or trans) to gain access to the transcription elongation complex. We used in vivo KMnO₄ footprinting to directly measure occupancy of the *hly* promoter and identify potential RNAP pause sites on the three different constructs described above. KMnO₄ reacts with thymine residues in melted DNA such as DNA opened in a transcription bubble (16). Cells transformed with plasmids containing either the intact *hlyCABD* operon or the Δ+126 to +137 or Δ+149 to +159 construct were pulsed for 15 s with KMnO₄. Some cultures were treated with rifampin to collect RNAP in open complexes on the promoter prior to KMnO₄ treatment. Plasmid DNA was isolated, quantified, and probed by primer extension on the nontemplate DNA strands.

Figure 5 shows KMnO₄-reactive thymines in the nontemplate strands of pSF4000, pWAM2240, and pWAM2245 from wild-type and *rfaH* null mutant *E. coli* during steady-state transcription (without rifampin) or following arrest of RNAP in the open complex at the *hlyCABD* promoter (with rifampin). Samples that were treated with rifampin reveal a highly reactive thymine in the open complex at the *hlyCABD* promoter (with rifampin). Samples that were not treated with rifampin reveal the steady-state level of promoter occupancy (+3) as well as a transcription-dependent reactive thymine just downstream of the JUMPStart se-

![FIG. 3. RNase protection analysis of steady-state hlyCABD mRNA levels in wild-type or rfaH null mutant E. coli. Cultures were grown to an OD₆₀₀ of 0.60. Extraction of total RNA, construction of antisense probes, and RNase protection analysis are described in Materials and Methods. (A) Map of hlyCABD operon antisense RNA probes. (B) Autoradiographs of radiolabeled antisense probes protected from RNase A and RNase T₁ digestion following hybridization to serial dilutions of total RNAs extracted from RZ8500 (rfaH) or WAM1925 (rfaH) harboring either pSF4000 (wild type [wt]) or pWAM2240 (Δ+126 to +137), or pWAM2245 (Δ+149 to +159). The amounts of total RNA probed are indicated below the lanes. The first lane in each panel represents 10% of the amount of antisense probe used in each hybridization reaction. (C) Histogram of relative levels of hlyCABD mRNA. The slopes of signal intensity as a function of total RNA probed for each strain were calculated and graphed relative to that for rfaH° E. coli harboring pSF4000. The error bars represent the standard deviations of results from two separate RNA extractions for each strain. The three probes do not contain equal amounts of radioactivity, and the film exposure times differ for each probe. Therefore, comparisons of relative intensities among the different constructs cannot be made.](http://jb.asm.org/content/179/12/3523.full.pdf)
FIG. 4. RNase protection analysis of steady-state hly leader and 5′/hlyC mRNA in wild-type or rfaH null mutant E. coli. See the legend to Fig. 3 for experimental details. (A) Map of hly leader-5′/hlyC mRNA antisense RNA probe. (B) Autoradiograph of radiolabelled antisense hly probe protected from RNase A and RNase T1 digestion following hybridization to serial dilutions of total RNAs extracted from RZ4500 (rfaH+1) or WAM1925 (rfaH) harboring either pSF4000 (wild type [w.t.]), pWAM2240 (Δ+126-+137), or pWAM2245 (Δ+149-+159). The amounts of total RNA probed are indicated below the lanes. The first lane represents 10% of the amount of antisense probe used in each hybridization reaction. (C) Histogram of relative levels of hly mRNA. The slopes of signal intensity as a function of total RNA probed for each strain were calculated and graphed relative to that for wild-type E. coli harboring pSF4000. The error bars represent the standard deviations of results from two separate RNA extractions for each strain.
sequences (+162). In Fig. 5A the lanes corresponding to pSF4000 with and without rifampin contain three times more DNA, and in Fig. 5B the lane corresponding to pSF4000 without rifampin contains two times more DNA, than the other lanes in order to demonstrate the locations and relative intensities of background signals compared to the +13 and +162 signals. All other lanes contain equal amounts of plasmid.

The data in Fig. 5 suggest several basic features of hlyCABD transcription. The +13 signal evident in the rifampin-treated samples confirms the location of the hlyCABD promoter identified by primer extension and deletion analysis in previous studies by our laboratory (20, 35). Quantitative comparisons of promoter signals on pSF4000 from wild-type and rfaH null mutant E. coli concurred with previous data which demonstrated that RfaH is not required for initiation of hly transcription. Deletion of the JUMPStart sequences did not alter the site or extent of open complex formation at the hly promoter in either the wild-type or rfaH null mutant E. coli. Finally, a combination of the rfaH null allele and the JUMPStart mutations did not alter the pattern of hly open complex formation.

In addition to providing information about the hly promoter in the context of the rfaH null allele and the JUMPStart deletions, the KMN6 footprinting technique was useful in identifying the highly reactive thymine at position +162 of pSF4000. This base represents an absolutely conserved T, three bases downstream of the conserved 5'GGTAG3' sequence within every JUMPStart sequence identified to date (Fig. 1). This T is modified by KMN6 only in the absence of rifampin treatment, when RNAP is allowed to proceed beyond transcription initiation. Footprints of the nontemplate DNA strands of pWAM2240 and pWAM2245 from wild-type and rfaH null mutant E. coli demonstrate that the T at position +162 is not modified when the JUMPStart subsequences are deleted. In addition, the rfaH null mutation has no significant effect on the appearance of the signal at +162, indicating that it is not required for the hyperreactivity. KMN6 footprinting of the template strand of either pSF4000, pWAM2240, or pWAM2245 from wild-type or rfaH null mutant E. coli RZ4500 did not reveal any transcription-dependent hypersensitive thymines in the JUMPStart region of hlyCABD DNA (18).

**DISCUSSION**

The noncoding regions of all RfaH-affected operons contain all or a portion of a 39-bp conserved sequence termed JUMPStart (15, 29). We and others have shown that RfaH is necessary for wild-type levels of promoter-distal mRNA in these operons, both in vivo and in vitro, supporting a model for the involvement of RfaH in transcription elongation (1, 2, 20). One of the major problems in our understanding of RfaH-dependent transcription elongation is the identity of the sequence or structure element(s) in the DNA or RNA that permits RfaH activity. The JUMPStart sequences represent potential cis-acting sites involved in RfaH-enhanced transcript elongation.

Nieto et al. (25) reported that removal of a 35-base sequence 2 kb upstream of an hlyCABD operon derived from a large, transmissible plasmid results in decreased hemolysin expression in vivo. Contained within that deletion was a portion of the conserved JUMPStart sequence (5'GGCGGTTAG3'). Further
ther work by that group demonstrated that removal of this sequence reduced transcription of the hlyCABD genes from a heterologous promoter in vitro (1). However, the location of this particular sequence relative to the native start of hlyCABD transcription was not demonstrated in their in vivo or in vitro systems.

In this study we examined precise deletion mutations of the JUMPStart consensus sequence within the untranslated leader sequence of a chromosomal hlyCABD operon, transcribed from the native hly promoter, for their effects on hemolysin expression from wild-type and rfaH null mutant E. coli. Our principal conclusions are that (i) like the rfaH null allele, the JUMPStart deletions result in reductions in hemolysin expression; (ii) the combination of JUMPStart deletions and the rfaH null mutation does not result in compounded reduction in hemolysin expression, and therefore the two are probably part of the same pathway; and (iii) presence of the JUMPStart subsequences confers transcription-dependent KMnO$_4$ hyperreactivity to a thymine in the nontemplate strand of hlyCABD DNA at position +162 (relative to the start of transcription).

Overall, our results are consistent with a role for the JUMPStart sequences in hlyCABD transcript elongation, lending further support to the hypothesis that RfaH and the JUMPStart sequences contribute to the same mechanism of transcriptional elongation.

Previous studies demonstrated that RfaH enhances hlyCABD mRNA levels, with a greater effect on promoter-distal hly mRNA than on promoter-proximal mRNA (20). The data shown in Fig. 3 and 4 demonstrate that the JUMPStart deletion mutants have an effect on hly leader and hlyCABD mRNA levels similar to that of the rfaH null allele. The combination of the JUMPStart deletions and the rfaH null mutation does not result in a compounded decrease in hlyCABD mRNA levels. These data strongly suggest that RfaH and JUMPStart do not act independently but instead influence the hlyCABD transcript via a common pathway.

We used the technique of in vivo KMnO$_4$ footprinting to identify open complexes and potential pause sites in the wild-type hlyCABD operon and in the deletion mutants expressed in wild-type and rfaH null mutants of E. coli (Fig. 5). Conservation of the JUMPStart sequence suggests that it is recognized by one or more host factors, including RNAS. Following treatment with rifampin to prevent initiation of transcription by RNAp, we identified a hyperreactive thymine at position +3 of the nontemplate strand, consistent with RNAP in open complex formation at the hlyCABD promoter. These data confirmed the previously mapped hlyCABD transcription start site on pSF4000 (20). The location and intensity of the open complex signal at +3 were the same on either the wild-type or JUMPStart-deleted hlyCABD constructs in wild-type or rfaH null mutant E. coli. On the basis of previous observations (20), our KMnO$_4$ footprint analyses, and RNA analyses, we conclude that RfaH and the JUMPStart sequences are not required for initiation of hlyCABD transcription.

KMnO$_4$ treatment of wild-type and rfaH null mutant E. coli in the absence of rifampin, which provides a steady-state profile of unstacked hlyCABD DNA, identified a hyperreactive thymine at position +162 of the nontemplate strand in the wild-type operon but not in the corresponding position of either JUMPStart mutant. A less reactive thymine at position +160 on the nontemplate strand is present in the KMnO$_4$ profiles of the wild-type and the JUMPStart-deleted constructs. The reactive thymine at base +157 is present in the KMnO$_4$ profiles of the wild type and the Δ+126 to +137 construct, but it is not present in the Δ+149 to +159 construct because this base is deleted in this mutant. One possibility to explain the footprinting and RNA data is that the JUMPStart sequences may signal RNAP to pause in elongation, perhaps in the region of position +160 to allow modification of the transcription complex to a more processive form. Removal of the JUMPStart sequences would inhibit this modification step, allowing the polymerase to move more rapidly through this early region (resulting in loss of KMnO$_4$ hyperreactivity) but more slowly past putative downstream pauses and terminators. A similar situation exists in the bacteriophage λ Q transcriptional elongation system, where mutations in the promoter-proximal Q binding site (qut) do not support an RNAP pause at positions +16 and +17, allowing Q to modify RNAP (16). RNAP stalled at the JUMPStart sequence would allow cis or trans antitermination factors, such as RfaH, to modify RNAP to an elongation-competent form. This model is consistent with the footprinting and RNase protection data from both wild-type and rfaH null mutant E. coli because RfaH is not required for polymerase to pause at the JUMPStart sequences (position +162 hyperreactive thymine); however, it would be required for further modification leading to an elongation-competent complex.

What is the role of RfaH in the JUMPStart-dependent elongation of hlyCABD transcription? RfaH could act like N, or like one of the E. coli Nus factors, in the λ or rrr antitermination systems. In λ N-mediated antitermination, the nut site (RNA) and several E. coli Nus proteins collectively help N locate its proper site on the surface of RNAP and maintain this physical association throughout subsequent transcription (10). For example, NusA allows N to capture RNAP transcribing the nut site, leading to a termination-resistant complex (36). Transcription through the E. coli rrr operons is also enhanced by a cooperative effect of several E. coli Nus factors that recognize signals resembling cis-acting sequences in the λ phage antitermination systems but do not interact with an N or Q homolog (3). RfaH could interact with RNAP transcribing the JUMPStart sequences. Deletion of RfaH would not inhibit interactions of RNAP with the JUMPStart sequences (RNA, DNA, or both), but RNAP would not be efficiently modified without RfaH present.

RfaH has significant amino acid sequence similarity with NusG (1, 18), a protein shown in E. coli to be involved in both transcriptional termination and antitermination (6, 7, 21, 22, 30, 31). The role of NusG in transcription by E. coli RNAP is controversial. Under certain conditions, NusG can enhance termination by rho (21, 31), or it can enhance antitermination by N at both rho-dependent (21) and rho-independent (21, 31) terminators. NusG has been shown to directly increase the rate of RNAP elongation, which may help RNAP get through pause sites and prevent termination by rho (7). NusG has also been shown to bind directly and selectively to rho and weakly to RNAP, which may bridge the rho-RNAP gap to enhance rho-dependent termination (21). The homology of RfaH with NusG suggests that RfaH may interact with known transcription factors; however, without knowledge of the functional domains of NusG, our model is speculative. Experiments are in progress to determine whether RfaH, like NusG, is involved in the antitermination systems of bacteriophage λ.

In conclusion, we identified multiple elements within the JUMPStart consensus sequence that are required for wild-type levels of hlyCABD mRNA in vivo. We also demonstrated a transcription-dependent unstacking of DNA within the hly leader region that depends upon the presence of both JUMPStart subsequences, which may be the result of RNAP paused at this site. Our data support a mechanism for enhanced elongation of transcription that requires both RfaH and the JUMPStart sequences.
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