Analysis of histidine-dependent antitermination in Bacillus subtilis hut operon

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ABSTRACT
We have previously shown that a positive regulator, HutP, of Bacillus subtilis hut operon is a RNA binding protein. Here, we report precise binding site of HutP in cis-regulatory region on hut mRNA and the role of hut in histidine-dependent antitermination of hut expression. Ethynitrosourea modification interference assay showed that four binding sites of HutP were found in the cis-regulatory sequences and were located at the stem and the internal loop of an antiterminator structure. In vitro transcription assay indicated that HutP suppressed transcription termination in the presence of histidine. These results suggest that HutP function as an antiterminator in response to the presence of histidine.

INTRODUCTION
The histidine utilization (hut) operon in Bacillus subtilis consists of a positive regulatory gene and five structural genes (1, 2). The hut structural genes are located far downstream of a promoter of the hut operon, while the positive regulatory gene, hutP, lies just downstream of the promoter (1). A stem-loop structure that functions as a terminator is present just upstream of the hut structural genes and is involved in controlling the expression of the hut structural genes (1). Expression of the hut operon is regulated by histidine induction, carbon catabolite repression, and amino acid repression (3, 4, 5).

Induction of the hut structural genes by histidine is positively regulated by the HutP protein, the product of the hutP gene (1). We recently reported that inducible expression of the hut structural genes is mediated by transcriptional antitermination at the stem-loop region (6). A secondary structure in hut mRNA, whose sequences overlap with the downstream terminator, is found in the regulatory region for the antitermination and is involved in controlling the antitermination (see Fig. 1B). In addition, we have recently shown that HutP binds hut mRNA, including the cis-acting regulatory sequences, in response to the presence of histidine (6). In this report, we determined the binding site of HutP in the cis-acting regulatory sequences. In addition, we demonstrated that HutP functioned as an antiterminator in response to the presence of histidine by use of transcription assay in vitro.

RESULTS AND DISCUSSION
To determine precise binding site of HutP in the cis-acting regulatory sequences on hut mRNA, a chemical modification interference assay was performed. ST4 RNA containing the regulatory region for antitermination was modified under denaturing conditions using ethynitrosourea (ENU) and incubated with HutP in the presence of histidine. After fraction with membrane filter, complexed and free RNAs were isolated and cleaved at the modified sites. Comparison of the band intensities between bound and free samples identified site of modifications that interfere with HutP binding (Fig. 1A). ENU-phosphate interference was observed at four positions: the 3'-phosphate of A483-U486, C496-A499, U504-A506, and A511-A513. These positions were located at the stem and the internal loop of the antiterminator structure (Fig. 1B). These results indicate that HutP binds putative antiterminator structure in the presence of histidine. Previous deletion analysis showed that possible stem-loop structure positioned between +483 and +520 plays an important role in the function of the antiterminator (6). Four binding sites of HutP were positioned in this stem-loop region.

Since HutP is the positive regulator for hut expression (1) and binds the antiterminator structure on hut mRNA in the presence of histidine, there is a possibility that HutP functions as an antiterminator in the presence of histidine. To examine this possibility, transcription assay with B. subtilis RNA polymerase α was performed in vitro (Fig. 2A). In the absence of HutP or histidine, transcription termination at the internal terminator was detected. However, transcription termination was suppressed in the presence of HutP and histidine. When the template DNA containing C495A mutation that inhibits binding of ST4 RNA to HutP was used, no suppression of transcription termination was observed. These results indicate that HutP inhibits transcription termination at the internal terminator.
Figure 1. ENU-footprinting analysis of the binding site of HutP on ST4 RNA. (A) 5'-end labeled RNA was treated with ethylnitrosourea (ENU), which ethylates phosphates in the RNA backbone, in the absence of HutP and mixed with HutP in the presence of histidine (10 mM). Complexed RNA with HutP and free RNA were separated by filtration. After strand scission at the modified sites, the RNA was fraction on 15% sequencing gel containing 8 M urea. Lane 1, guanine-specific sequence obtained with RNase T1; lane 2, adenosine-specific sequence obtained with RNase U2; lane 3, RNA hydrolysis ladder; lanes 4 to 6, only RNA; lane 7, bound RNA to HutP; lane 8, free RNA. (B) The sites of modifications which interfere with the binding of HutP are shown by arrow heads on putative antiterminator on hut mRNA.

by binding to the antiterminator structure on hut mRNA in the presence of histidine.

Since HutP was shown to be a hexamer in the presence and the absence of histidine (6), it was estimated that hexamer HutP binds the antiterminator structure in the presence of histidine. Our data showed that HutP functioned as the antiterminator in the presence of histidine. Since the sequences of antiterminator is overlapped with the downstream terminator, it is suggested that binding of HutP to the sequences of antiterminator stabilizes the structure of antiterminator which then inhibits formation of a downstream terminator structure.

REFERENCES