Specific Binding of Escherichia coli Ribosomal Protein S1 to boxA Transcriptional Antterminator RNA

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We show that ribosomal protein S1 specifically binds the boxA transcriptional antterminator RNAs of bacteriophage λ and the Escherichia coli ribosomal RNA operons. Although S1 competes with the NusS-S10 anttermination complex for binding to boxA, it does not affect anttermination by the N protein in vitro, and its role, if any, in rRNA synthesis is still unknown.

Translation of mRNA in Escherichia coli suppresses the transcriptional termination activity of termination factor Rho because the translating ribosomes prevent Rho from binding the nascent RNA (reviewed in reference 5). Untranslated transcripts, such as those synthesized from the ribosomal RNA (rrn) operons, are more readily accessible to Rho. The rrn operons are, however, transcribed without premature termination of transcription. The observation that insertion of strong Rho-dependent terminators within rrn caused only a small reduction in the transcription of downstream sequences indicated that a mechanism exists that renders the rrn operons resistant to the action of Rho (17). Later experiments suggested that this mechanism is transcriptional anttermination (1).

Transcriptional anttermination has been best characterized in bacteriophage λ (for a review, see reference 8). The N protein is able to modify RNA polymerase so that it becomes resistant to both Rho-dependent and Rho-independent terminators (15, 30). A cis-acting element called the nut site (22) must be transcribed into RNA for N to function (11, 19). The nut site consists of two functional elements, boxA and boxB. boxB RNA can form a 15-nucleotide stem-loop that binds N (4, 16, 19). boxA RNA is a 12-nucleotide sequence 5′ to boxB that interacts with host factors (16, 20). The host factors involved in N-mediated anttermination are NusA, NusB, NusG, and ribosomal protein S10 (NusE) (6, 27). It is thought that N, the Nus factors, and the nut site form a ribonucleoprotein complex that stays associated with elongating RNA polymerase and directs the enzyme to transcribe through termination signals (19).

Anttermination in the rrn operons depends on boxA sequences that are closely related to the boxA elements of λ nut sites (13). Moreover, rrn boxA RNA has been shown to bind a heterodimer of NusB and S10 (14, 20). NusB was further implicated in rrn anttermination by experiments showing that NusB is important for RNA synthesis in vivo (23) and that a NusB-depleted extract is unable to support anttermination in vitro (25). However, anttermination in the rrn operons is known to differ from N-mediated anttermination in three major ways: first, the bacteriophage λ N protein is not involved in rrn anttermination; second, rrn boxA is capable of supporting anttermination in the absence of boxB or any other RNA sequence (2); third, an unidentified factor(s) is required for anttermination in vitro in the rrn system but not in the λ system (25). The present study was initiated to attempt to identify this missing factor(s) and other proteins that interact with boxA RNA.

To detect E. coli proteins that bind boxA, S100 extract (7) was incubated in buffer (10 mM HEPES [pH 7.3], 10 mM ammonium sulfate, 15 mM potassium chloride, 0.5 mg of bovine serum albumin/ml, 50 μg of tRNA/ml) with a 35-nucleotide radiolabeled RNA (AGGGAAAGUUCACUGCUUU UAAACUUUUAGUCGA) containing the 12-nucleotide rrn boxA element (underlined), or boxA inserted in the reverse orientation as a control, and was run on a nondenaturing 10% polyacrylamide gel containing 0.05 M KCl, and fractions containing the boxA RNA probe containing boxA was not (Fig. 1, compare lanes 1 to 4 with lanes 5 to 8). This shifted band was not a consequence of NusB and S10 binding the probe, as this band still formed when antibody against NusB was used to deplete the extract of NusB (data not shown). Furthermore, the band had a mobility different from that of the band that appeared when purified NusB and S10 were incubated with the probe (see Fig. 5).

Column chromatography was used to purify and identify the protein that was retarding the mobility of boxA in the mobility shift experiment (Fig. 2). S100 extract was first passed over a DEAE-cellulose column (Whatman DE52), and the column was washed with buffer (10 mM Tris-acetate [pH 7.8], 14 mM magnesium acetate, 1 mM dithiothreitol) containing 0.14 M KCl. The protein with boxA-binding activity was then eluted with buffer containing 0.25 M KCl. Fractions containing the protein were pooled (Fig. 2, lane 3) and loaded onto a phenyl-Sepharose column. The protein was eluted with buffer containing 0.05 M KCl, and fractions containing the protein were again pooled (Fig. 2, lane 4) and loaded onto a poly(U)-agarose column (Pharmacia Biotech). The protein remained bound when this column was washed with 1 M KCl and was eluted with 6 M urea. After these purification steps, only one major polypeptide with an apparent molecular mass of 70 kDa and a minor, 60-kDa polypeptide were evident on a sodium dodecyl sulfate-polyacrylamide gel stained with Coomassie blue (Fig. 2, lane 5). Gel purification and renaturation (10) of

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To identify the 70-kDa protein, its N-terminal sequence was determined for 19 amino acids, providing the identity of the amino acids at all positions except 14 and 16. This sequence was identical to that predicted for ribosomal protein S1 (Fig. 1). The known sequence of ribosomal protein S1. xxx, unknown amino acid.

In order to characterize further the specificity of the interaction between S1 and boxA, gel mobility shift experiments were performed with mutant boxA probes (Fig. 4 and Table 1). Certain point mutations in boxA at positions 1, 5, and 7 did not affect the amount of S1 required to shift the mutant probe (Table 1; compare, e.g., Fig. 4A lanes 1 to 4 with lanes 5 to 8). A second class of mutations at positions 2, 4, 6, and 11 substantially affected the interaction between S1 and boxA so that approximately nine times more S1 was required to shift the mutant probe (Table 1; compare, e.g., Fig. 4A lanes 1 to 4 with lanes 9 to 12). A third class of mutations at positions 3, 8 plus 10, 9, and 12 affected the interaction between S1 and boxA so that at least 30 times as much S1 was required to shift the mutant probe (Table 1; compare, e.g., Fig. 4A lanes 1 to 4 with lanes 13 to 16). An almost undetectable amount of probe containing reverse boxA was shifted even at the highest concentration of S1 used (Fig. 4D, lane 16). Approximately equal amounts of S1 were needed to shift probes containing the nutR boxA sequence and wild-type rm boxA (Fig. 4E, compare lanes 1 to 4 with lanes 5 to 8). Thus, the interaction of S1 with

is essential for the growth of *E. coli* (12), presumably because of the role of S1 in translation, participation by S1 in other processes might easily have escaped attention. S1 is an essential subunit of the replicase of bacteriophage Qβ (29). Recently, S1 has also been shown to be complexed with NusA and recombination protein β of phage λ, although the significance of this complex is unclear (28). Nevertheless, we have found that NusA alone does not bind *rrn boxA* or *nut* site RNA in a gel mobility shift assay (16, 20) and does not affect the binding of S1 to *boxA* RNA (data not shown).

![FIG. 1. A factor in a crude *E. coli* extract binds *rrn boxA* RNA. The indicated amount of S100 extract was incubated with radiolabeled *rrn boxA* RNA (lanes 1 to 4) or reverse *boxA* RNA (lanes 5 to 8) and electrophoresed on a nondenaturing polyacrylamide gel. WT, wild type.](image1)

![FIG. 2. Purification of a 70-kDa protein that binds *boxA* RNA. Fractions containing *boxA*-binding activity were pooled after DE52 (lane 3), phenyl-Sepharose (lane 4), and poly(U)-agarose (lane 5) column chromatography and were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis and stained with Coomassie blue. Lane 1 contains protein molecular mass standards (97.4, 66.2, 45.0, 31.0, and 21.5 kDa from top to bottom), and lane 2 contains *E. coli* S100 extract.](image2)

![FIG. 3. Comparison of the N-terminal sequence of the 70-kDa protein with the known sequence of ribosomal protein S1. xxx, unknown amino acid.](image3)

![FIG. 4. Comparison of the N-terminal sequence of the 70-kDa protein with the known sequence of ribosomal protein S1. xxx, unknown amino acid.](image4)

![TABLE 1. Binding of ribosomal protein S1 to mutant *boxA* RNAs](table1)
or RNA boxA RNA is highly specific and could potentially play a role in λ and/or rrn antitermination.

To determine if S1 could enter a ribonucleoprotein complex with NusB, S10, and RNA boxA, a gel mobility shift experiment was performed in which purified NusB, S10, and S1 were incubated at various concentrations with RNA containing boxA (Fig. 5). Probe shifted by S1 had a lower electrophoretic mobility than probe shifted by the NusB-S10 complex (Fig. 5A, compare lanes 2 and 5). As S1 was added in increasing amounts to reaction mixtures containing NusB and S10, the NusB-S10-RNA complex disappeared and no supershifted band was observed (Fig. 5A, lanes 5 to 8). Thus, S1 apparently competes with NusB and S10 for binding to boxA RNA. This result is consistent with our observations that the nucleotides most important for binding S1 (Table 1) are also important for binding NusB and S10 (20) (Table 1). In a similar experiment, addition of increasing amounts of NusB-S10 to reaction mixtures containing S1 decreased the amount of S1-RNA complex without producing a supershifted complex (Fig. 5B, lanes 2 to 6), again demonstrating that NusB-S10 and S1 cannot simultaneously bind boxA RNA.

Our binding data (Fig. 5) indicated that the affinity of RNA boxA for S1 is at least 200 times greater than its affinity for the NusB-S10 complex. This raised the possibility that S1 might be an inhibitor of antitermination. Nevertheless, adding purified S1 did not inhibit RNA boxA-mediated antitermination in reactions containing crude E. coli extract (25), nor did it make possible rrn antitermination in vitro when it was added to reactions containing purified Nus factors (24). Therefore, the significance of the specific interaction we have described between RNA boxA and ribosomal protein S1 is still unclear.

The existence of an E. coli inhibitor of N-mediated antiter-

FIG. 4. Mutations in boxA affect its ability to bind ribosomal protein S1. Various concentrations of S1 were incubated with radiolabeled RNAs containing wild-type (WT) or mutant boxA as indicated, and the reaction mixtures were electrophoresed on nondenaturing polyacrylamide gels.
mination that binds boxA has been predicted by Patterson and colleagues on the basis of genetic experiments with deletion and point mutations in boxA (21). To determine whether S1 could be this inhibitor, we first compared the strength of binding between S1 and a probe containing hrn boxA with that of S1 and a probe containing a λ nut site (boxA + boxB) (Fig. 6). S1 bound the nut site probe, although approximately eight times as much S1 was required to shift the nut site probe as to shift a similar amount of the hrn boxA probe (Fig. 6, compare lanes 2 to 5 with lanes 7 to 10), raising the possibility that boxA and S1 might play a role in the processing of RNA.

Another possibility is provided by the recent observation that the λ N protein can repress the translation of its own mRNA in a process that requires the nut site but appears to be independent of NusA, NusB, and S10 (31). The boxA16 mutation, which alters the fifth nucleotide (underlined) of λ boxA (CGCUCUUACACA), prevents antitermination of transcription but not repression of translation, whereas the boxA5 mutation, which alters the second nucleotide of λ boxA (CCGU CUUACACA), prevents both (31). Interestingly, nucleotides 2 and 5 of boxA are both important for the binding of NusB and S10 (20) (Table 1), whereas only nucleotide 2 is important for the binding of S1 (Fig. 3 and Table 1). Translational repression may therefore involve a complex containing the ribosome in which the binding of N to boxB and the binding of ribosomal protein S1 to boxA at the nutL site somehow prevent or aid binding of the ribosome to the AUG initiator codon of the N gene located not far downstream of nutL.

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