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Identification of a Heat Shock Promoter in the *topA* Gene of *Escherichia coli*

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The transcriptional activity of the *topA* gene which codes for topoisomerase I was examined. An in vitro assay determined that the P1 promoter was dependent on the σ^{32} subunit of RNA polymerase. The transcriptional activity of the four *topA* promoters was examined by nuclease S1 mapping of the transcripts during a heat shock. This σ^{32}-dependent promoter was shown to function as a heat shock promoter, although topoisomerase I is not a heat shock protein. A possible method of compensation of transcription activity by the other promoters to maintain the level of topoisomerase I during heat shock is proposed.

Escherichia coli topoisomerase I catalyzes the interconversion of topoisomers of DNA via the concerted breaking and rejoining of a single DNA strand (see references 9 and 26 for reviews). Topoisomerase I activity, along with gyrase activity, has a direct influence on the superhelicity of the chromosome. The level of DNA supercoiling is controlled by the balance of the two opposing enzyme systems. Expression of many genes has been shown to be influenced by the level of topoisomerase activity (6, 7, 19, 24, 25). Indeed, mutations in *E. coli* which delete the gene encoding topoisomerase I (*topA*) are only viable in the presence of compensatory mutations which often map in subunits of DNA gyrase (*gyrA*, *B*) (6, 18; C. S. Laufer and R. E. Depew, Fed. Proc. **43**:1542, 1984).

Expression of both the topoisomerase I and gyrase genes is regulated by the level of supercoiling (16, 22). It has been suggested that homeostatic regulation is involved in the expression of these genes and global regulation of DNA supercoiling (16). More recently, it was demonstrated that localized supercoiling can be generated by transcription and that topoisomerases are required for modulation of such transcription-driven supercoiling in the elongation step of transcription (15, 21, 27). The level of DNA supercoiling has been observed to change in response to various environmental and growth conditions (1, 28); hence, the genes which control the level of DNA supercoiling might be expected to respond to some or all of these conditions.

Four promoters have been identified and sequenced upstream of *topA*. These have been cloned in front of the galactokinase gene (*galK*) such that galactokinase activity is a measure of promoter activity (23). The promoter (P1) closest to the ATG start codon has a sequence that does not align well with the consensus σ^{70} promoter sequence. A fusion of the *topA* P1 promoter to galactokinase was used in an in vitro assay to determine the sigma-factor requirement of P1. By using an in vitro transcription assay, the *topA* P1 promoter was determined to be σ^{32} dependent.

The σ^{32} subunit of RNA polymerase is required for the expression of heat shock proteins (11), but the *topA* gene product is not a heat shock protein. Nuclease S1 mapping of the transcripts from the *topA* promoters during a heat shock was performed. The P1 promoter showed a strong increase during heat shock. Data from the S1 mapping experiments indicate that a compensatory decrease in upstream promoters may account for the relatively stable level of topoisomerase I during a temperature upshift.

MATERIALS AND METHODS

Polymerase purification and plasmids. RNA polymerase containing σ^{32} was purified as described previously (14). Plasmids containing *topA* promoters fused to *galK* have been described before (23). Plasmid pBDR1 contains P1, P2, P3, and P4. Plasmid pBDR2 contains P1. Plasmids were purified by CsCl gradient centrifugation for use in transcription assays and S1 mapping.

Transcription and nuclease S1 mapping. Transcription reactions using purified $E\sigma^{70}$ or $E\sigma^{32}$ polymerase were performed under conditions essentially as described by Chen and Richardson (4). The template DNA, pBDR2, was linearized by digestion with *Bam*HI prior to transcription. Transcription from the individual *topA* promoters was measured by S1 nuclease mapping as described previously (2). RNA was isolated from C600 *galK recA* containing pBDR1. Samples were taken from cells in logarithmic growth at 30°C and at 5, 10, and 15 min after shifting to 42°C. Purified RNA (50 μg) and yeast tRNA (50 μg) were hybridized to an excess of an *Eco*RI-*Bam*HI restriction fragment, containing the four promoters, that had been end labeled with ^{32}P at the *Bam*HI site. This site is in the vector portion of the construct so the observed transcription was derived from the plasmid rather than the chromosome. Samples were denatured and hybridized at 45°C for 4 h prior to treatment with S1 nuclease. Protected DNA fragments were separated on a 5% denaturing polyacrylamide gel and visualized by autoradiography.

RESULTS

The P1 promoter sequence is similar to but contains several mismatches with the σ^{32} consensus (5) (Table 1). To confirm the presence of a σ^{32}-dependent promoter, in vitro transcription with purified components was performed (Fig. 1). Plasmid pBDR2 was linearized with *Bam*HI and transcription was performed with purified RNA polymerase containing either σ^{32} or σ^{70}. Transcription with σ^{70} holoenzyme produces a transcript of approximately 106 bases

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TABLE 1. Sequence alignments of *topA* promoters

Promoter	−35 region	Spacing	−10 region
P4	TTGCGT	N_{16}	TACAGT
P3	GTGACG	N_{18}	TAGATT
P2	TTGACA	N_{19}	TGCTAT
σ^{70} consensus	TTGACA	N_{17}	TATAAT
P1	ACAAGGGGTTGAT	N_{16}	GTCCATATC
σ^{32} consensus	TCTCNCCCTTGAA	N_{13-17}	CCCCATNTA

which corresponds in size to the RNA1 transcript for plasmid replication (3). Transcription with σ^{32} holoenzyme generates a transcript of approximately 110 bases corresponding to P1. These results indicate that σ^{32}, and not σ^{70}, is required for transcription of P1 and indicate that no auxiliary factors are required for transcription of this promoter by the σ^{32} holoenzyme. The σ^{32} dependence of P1 was confirmed by transcription inhibition by anti-σ^{32} monoclonal antibodies as described previously (13, 14) (data not shown). The P1 promoter was also shown to be insensitive to inhibition by anti-σ^{70} monoclonal antibodies.

The *topA* P1 promoter was examined to determine whether it is a heat shock promoter. Temperature upshift in the temperature-sensitive σ^{32} mutant *rpoH165* shows no significant increase in transcription from P1 as measured by galactokinase activity (data not shown). The temperature-sensitive σ^{70} mutant *rpoD800* showed a high stimulation under the same conditions, further indicating that P1 is a functional σ^{32} promoter. S1 mapping was also performed to monitor the level of transcription at various times after a shift from 30 to 42°C. Figure 2 shows that there is a large increase in transcription from P1 after the temperature shift, characteristic of the heat shock response. The large increase is transitory and returns to a slightly elevated level by 15 min after the temperature increase. In contrast, the *topA* P4 promoter shows a transitory decrease in transcription. The

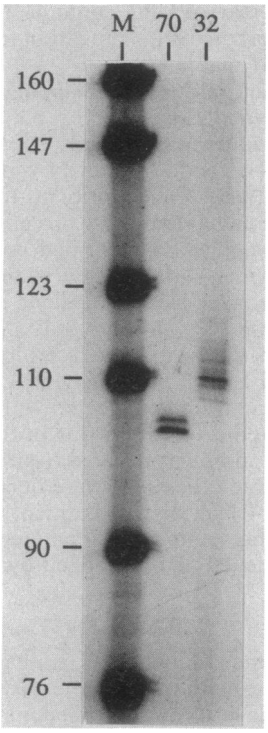

FIG. 1. Transcription using purified RNA polymerase. Plasmid pBDR2 containing *topA* P1 was linearized with *Bam*HI prior to transcription as described in Materials and Methods. ^{32}P-labeled transcripts from reactions containing Eσ^{70} or Eσ^{32} are shown. Labeled DNA fragment marker sizes are indicated in bases.

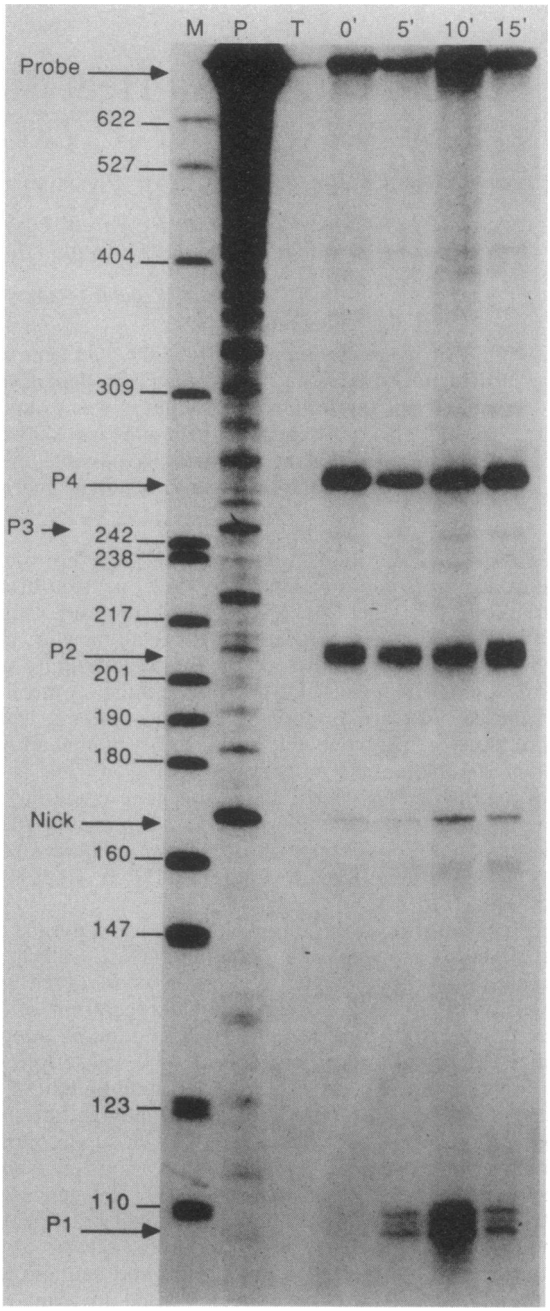

FIG. 2. S1 mapping of *topA* promoter region. S1 nuclease mapping of promoter transcripts was performed as described in Materials and Methods. RNA samples were taken immediately prior to and at 5, 10, and 15 min after shifting to 42°C as indicated at the top. Yeast tRNA (lane T) was substituted for *E. coli* RNA as a control. The probe is shown in lane P. Molecular weight markers derived from *Msp*I-cut pBR322 are shown (lane M), with the number of bases indicated. The expected positions of the *topA* promoters P1 through P4 are indicated by arrows.

P2 level shows an increase in transcription after 15 min at 42°C. By this compensatory mechanism of temporal regulation of multiple promoters, the overall level of transcription of *topA* may remain relatively constant during heat shock. The P3 promoter signal was virtually undetectable. The reason for this is unclear since the reported activity with RNA from C600 *galK* grown at 37°C was relatively high (23). The strain used for nuclease S1 mapping (Fig. 2) was also *recA*. Some influence of this mutation may be responsible for the observed differences in activity. The inconsistency in activity could also be due to differences in the assay used (primer extension versus nuclease S1 mapping).

The topoisomerase I content was examined during heat shock to determine whether the level increased. Immunoblot analysis of whole-cell lysates (data not shown) shows that the level of topoisomerase I remains fairly constant during the heat shock. In contrast, the level of σ^{32} shows a transitory increase during the heat shock. The transitory increase in σ^{32} has been shown previously (14, 20) and is indicative of the heat shock response. The lack of increase in the level of topoisomerase I during heat shock does not rule out the possibility of its synthesis being increased during heat shock if degradation also increases. Analysis of two-dimensional gels indicates that synthesis of numerous proteins increases during heat shock (17). Topoisomerase I is not included on this list. Therefore, the synthesis and level of topoisomerase I do not appear to increase during heat shock.

DISCUSSION

The level of supercoiling of the chromosome has the potential for being a global mechanism of transcriptional regulation. It is not surprising, therefore, that topoisomerase is a highly regulated gene product. In addition to the dependence of *topA* promoter activity upon supercoiling described previously (23), the results presented here indicate that the transcription originating from the various promoters is affected by the heat shock response. The *topA* P1 promoter was shown to be a σ^{32}-dependent heat shock promoter, while the P4 promoter shows a transitory decrease in activity during heat shock. Although the sigma dependence of *topA* P2, P3, and P4 was not determined, Table 1 shows that P2, P3, and P4 have reasonable matches to the σ^{70} consensus using nonoptimal spacing.

The effect of heat shock upon supercoiling levels is unclear. It has been shown that a temperature shift between 17 and 37°C affects the level of supercoiling (10); however, the response is not immediate. Transcription of a wide variety of genes has been shown to be dependent on the level of supercoiling (12, 26; Jovanovich et al., unpublished data). The heat shock response involves only a relatively small set of proteins. While we do not rule out localized changes in DNA supercoiling, it seems logical that the cell would need to maintain a constant superhelicity during heat shock. This should require constant levels of topoisomerase I and DNA gyrase, the proteins involved in the regulation of supercoiling. Despite the fluctuations in promoter activity during heat shock, the level of topoisomerase I is relatively constant. The increase in P1 transcription can easily be explained by a well-characterized increase in σ^{32} activity during heat shock (8, 20). The decrease in P4 activity could be due to a relative decrease in σ^{70} activity during heat shock. This suggests that there is a compensation of the various transcriptional activities of the *topA* promoters. Given these results, it would be interesting to determine what effect heat shock has on the transcription and synthesis of DNA gyrase since the ratio of topoisomerase I to DNA gyrase is important for maintaining the level of supercoiling (26).

In summary, the results presented here identify a σ^{32}-dependent promoter in the *topA* promoter region. The σ^{32}-dependent promoter functions as a heat shock promoter; however, no increase in topoisomerase I levels are seen. Nuclease S1 analysis of the promoters transcribing *topA* indicate a possible compensatory mechanism for maintaining topoisomerase I levels during a heat shock. These results also raise the question of determining how many other non-heat shock proteins contain σ^{32} promoters.

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