

Electron microscopy mapping of Escherichia coli RNA polymerase-binding sites on plasmids from thermophilic bacteria

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Abstract

The binding sites of Escherichia coli RNA polymerase to plasmid DNA from extremely thermophilic bacteria have been mapped by electron microscopy. Templates used in these studies included plasmids pTF62 (from Thermus flavus AT62) and pTT8 (from T. thermophilus HB8) and also hybrid molecules constructed by ligation of these plasmids to pBR322. Although the affinity of the enzyme for heterologous DNA was about one-third of that for pBR322, it was possible to localize preferred binding sites on pTF62 and pTT8. Six binding sites were identified in pTT8, mapping close to 7, 28, 47, 61, 65, and 81 map units (one unit being equal to 1% of the length of the DNA). Seven such regions located at 3, 27, 48, 60, 67, 81, and 86 map units were found in pTF62. RNA polymerase binding sites found in pBR322 coincided with promoters identified previously by electron microscopy analysis of transcriptional complexes prepared in vitro. These data indicate that E. coli RNA polymerase binds preferentially to specific sequences in plasmids from thermophilic bacteria, suggesting possible promoter locations in these plasmids.