Note

Electron Microscopic Mapping of *Escherichia coli* RNA Polymerase Binding Sites on Tetracycline Resistant Plasmid pNS1

Norihisa NOGUCHI, Kazuo SHISHIDO,*
Tadahiko ANDO** and Megumi KONO

Department of Microbiology, Tokyo College
of Pharmacy, 1432-1 Horinouchi, Hachioji-shi,
Tokyo 192-03, Japan

*Laboratory of Natural Products Chemistry,
Tokyo Institute of Technology, Nagatsuta,
Yokohama, Kanagawa 227, Japan

**Department of Microbiology, The Institute
of Physical and Chemical Research, 2-1
Hirosawa, Wako-shi, Saitama 351, Japan

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Tetracycline resistant (TC^r) plasmid pNS1¹⁾ (2.55 Mdaltons) have been constructed from parental pTP-5 (2.9 Mdaltons) originating from *Staphylococcus aureus*.²⁾ pNS1 can replicate and are stably maintained in *Bacillus subtilis*. The copy number of pNS1 in *B. subtilis* is low under the condition without TC, whereas upon addition of TC, the copy number is elevated to about 300. This property of pNS1 seems very likely to be advantageous for its use as a cloning vector in *B. subtilis*. Fine restriction map of pNS1 was made and the TC^r region and sequence essential for replication (or maintenance) were estimated through construction of still smaller plasmids.

Here in order to elucidate the genome organization on pNS1, we attempted the mapping of RNA polymerase binding sites using *Escherichia coli* RNA polymerase because it is well known that genetic information of staphylococcal drug resistant plasmids is expressed in *E. coli*^{3~5)} as well as *B. subtilis*.⁶⁾ The mapping of the RNA polymerase binding sites was performed electron microscopically using the linearized pNS1 DNA after digestion with three restriction endonucleases which have a single cleavage site on pNS1 DNA. Five binding sites were identified and four sites out of the five were mapped on the essential region for autonomous replication of the drug resistant plasmid.

E. coli RNA polymerase was incubated with the *Hpa* II and *Kpn* I-generated unit length linear DNAs under low salt conditions (50 mM KCl) and with the *Xba* I-generated unit length linear DNA under high salt condition (150 mM KCl). Taking the *Xba* I site as a zero point, the *Kpn* I and *Hpa* II sites are located at map positions (Mdaltons) 0.52 and 2.03, respectively (see Fig. 2). Three sorts of RNA

polymerase-DNA complexes were fixed by glutaraldehyde as described by Lescure *et al.*⁷⁾ and were examined by electron microscopy. To construct histograms of the distribution of the RNA polymerase binding sites, 512, 288 and 245 molecules of three linearized DNAs after digestion with *Hpa* II, *Kpn* I and *Xba* I were scored, as shown in Fig. 1-A, B and C. On average, about two RNA polymerase molecules were bound to each DNA molecule. Electron micrographs of the RNA polymerase-DNA complexes are shown in Fig. 1-D, E and F.

It was proved from the experimental results shown in Fig. 1 that five specific RNA polymerase binding sites are present on pNS1. They are located at map positions 0.13 ± 0.06 , 0.61 ± 0.048 , 1.15 ± 0.062 , 1.66 ± 0.061 and 2.19 ± 0.045 from the *Xba* I cleavage site with almost similar frequency. Differences in the localization of the RNA polymerase binding sites between the low and high salts conditions were not so significant⁸⁾ (compare Fig. 1-A and B with Fig. 1-C).

Figure 2 shows the map of the RNA polymerase binding sites from the point of view of the genome organization on pNS1. Four sites out of the five are located in the essential region. Three binding sites at positions 0.13 ± 0.06 , 0.61 ± 0.048 and 1.15 ± 0.062 are mapped in the TC^r region (0.08 to 1.17). One binding site at position 0.13 ± 0.06 is located close to the boundary between the two regions essential for TC resistance and replication (or maintenance). We have already observed that the deletion derivatives which have deletions in the map region from 1.15 to 1.17 exhibit decreases of the level of drug resistance and copy number in comparison with parental pNS1,¹⁾ and the other derivative which lacks the binding site region at 1.15 ± 0.062 completely is sensitive to TC.¹⁾ These results suggest that the site at 1.15 is the promotor of the gene essential for TC resistance and the TC^r gene is related to regulation of the copy number. The presence of a RNA polymerase binding site also in the middle of the TC^r region seems to suggest that two sorts of proteins are encoded on the TC^r region.

To elucidate the molecular biology of TC^r plasmid pNS1, DNA sequencing and analysis of gene products are in progress.

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FIG. 1. Histograms of the Distribution of *E. coli* RNA Polymerase Binding Sites on pNS1 and Electron Micrographs of RNA Polymerase-DNA Complexes.

pNS1 was digested with *Hpa* II, *Kpn* I and *Xba* I according to the instructions of Bethesda Research Lab. Inc. (BRL). Three sorts of unit length linear DNA (0.15 μg each) were incubated at 37°C for 5 min with *E. coli* RNA polymerase holoenzyme purchased from BRL (6 units) in 20 μl of buffer containing 40 mM Tris-HCl (pH 7.9), 10 mM MgCl₂, 0.1 mM EDTA, 10 μg bovine serum albumin and the indicated concentrations of KCl. The RNA polymerase-DNA complexes were spread for electron microscopy by the technique of Dubochet *et al.*,⁹⁾ after glutaraldehyde fixing.⁷⁾ Electron micrographs were taken at a magnification of 20,000 with an electron microscope, JEM 100CX. The photographs were projected, and the length of the DNA molecules and the RNA polymerase binding positions were measured with a graphics calculator (Numonics Model 1250). A, B and C are histograms of the distribution of the RNA polymerase binding sites. D, E and F are electron micrographs. A and D are of the *Hpa* II digest; B and E, the *Kpn* I digest; C and F, the *Xba* I digest. The *Xba* I site is assigned to the zero point and the enzyme cleavage sites of *Hpa* II and *Kpn* I are indicated by arrows. Bars are 0.5 μm.

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FIG. 2. Map of *E. coli* RNA Polymerase Binding Sites from the Point of View of the Genome Organization on pNS1.

Restriction endonuclease cleavage sites of *Xba* I, *Kpn* I, *Hpa* II and *Hind* III are presented in the figure. The *Xba* I site is taken as the zero point. The RNA polymerase binding sites are indicated by arrows and the width of arrows is the standard deviation. Genetic maps of pNS1 were cited from our previous work.¹⁾ The boundary ({) between the two regions essential for TC resistance and replication was estimated from the results of the construction of a composite plasmid between pNS1 and chloramphenicol resistant plasmid pTP-4²⁾ (unpublished observation).