Simian Virus 40 Promoters Direct Expression of the Tetracycline Gene in Plasmid pACYC184

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Insertion of *Hind*III DNA fragments into the *Hind*III site of plasmid pACYC184 destroys the promoter of the plasmid tetracycline resistance gene and causes *Escherichia coli* cells harboring recombinant plasmids to be tetracycline sensitive and chloramphenicol resistant. The *Hind*III-C DNA fragment of simian virus 40 contains the two virus promoters and the virus origin of replication. We report the isolation of recombinant plasmids that contained the simian virus 40 *Hind*III-C DNA fragment at the *Hind*III site but were capable of conferring tetracycline resistance to *E. coli* cells. The viral promoter sequences contained in the *Hind*III-C fragment presumably replaced the inactivated tetracycline resistance gene promoter sequences and enabled transcription of the tetracycline resistance gene.

The simian virus 40 (SV40) genome contains two major promoter regions. The first region, located between the viral origin of replication and nucleotide number 5,154, contains promoter sequences for the early genes, and the second set of promoter sequences, located between the viral origin of replication and nucleotide number 1,029, directs expression of the late genes (7). Both sets of promoter sequences and the viral origin of replication are contained in the 1.101-kilobase *Hind*III-C fragment of SV40 DNA (4). We were interested in cloning the SV40 *Hind*III-C fragment into the homologous restriction site of plasmid pACYC184. The *Hind*III site of pACYC184 is located in the promoter sequences of the tetracycline resistance gene. Insertion of DNA fragments into this site destroys the promoter of the tetracycline resistance gene and causes *Escherichia coli* cells harboring recombinant plasmids to be tetracycline sensitive and chloramphenicol resistant (11, 13). We report the isolation of tetracycline-resistant recombinant plasmids containing the SV40 *Hind*III-C fragment. Tetracycline resistance was expressed regardless of the orientation of the SV40 *Hind*III-C fragment relative to the tetracycline gene. This resistance was not due to the size of the C fragment, as insertion of the similarly sized SV40 *Hind*III-B fragment (1.169 kilobases) did not confer tetracycline resistance. We concluded that the promoter sequences of SV40 contained in the *Hind*III-C fragment replaced the inactivated tetracycline promoter sequences, thereby enabling efficient transcription of the tetracycline gene.

Plasmid pACYC184 was kindly provided by

M. Stinski, University of Iowa. All plasmids were grown in *E. coli* HB101 cells in LB broth (1% tryptone, 0.5% NaCl, 0.5% yeast extract, and 0.2% MgCl₂) at 37°C. SV40 DNA was kindly provided by M. J. Tevethia, The Pennsylvania State University. Plasmid DNA was isolated as previously described (5).

Initially we attempted to clone the SV40 *Hind*III-C fragment by shotgun-cloning *Hind*III-digested SV40 DNA into the *Hind*III site of pACYC184. Equimolar amounts of *Hind*III-digested SV40 and pACYC184 DNAs were ligated and transfected into calcium-treated strain HB101 cells (6), and the plasmid DNA from the resulting tetracycline-sensitive colonies was analyzed for SV40 inserts by gel electrophoresis. We found several colonies that contained either SV40 *Hind*III-B or -D inserts, but none that contained *Hind*III-C inserts.

We then enriched for the *Hind*III-C fragment by isolating the B and C fragments from a 1.4% agarose gel. It was necessary to isolate both the B and C fragments because they cannot be separated efficiently on agarose gels, since they differ in length by only 68 base pairs. Isolated B and C fragments were then ligated to *Hind*III-digested pACYC184 and transfected into HB101 cells. Gel electrophoresis analysis of plasmid DNA from 28 tetracycline-sensitive colonies revealed 23 *Hind*III-B-containing colonies and 5 colonies containing both B and C fragments (data not shown). Again, no *Hind*III-C fragment inserts were detected.

A third strategy for cloning the SV40 *Hind*III-C fragment was then employed. Recombinant plasmid pSV65, which contains both *Hind*III-B

FIG. 1. Analysis of DNA plasmids from tetracycline-resistant bacteria. DNA samples were digested with *Hind*III and electrophoresed through a 6.5% polyacrylamide gel. DNA bands were visualized under UV light after being stained with ethidium bromide. Lanes 1, 5, and 9 contain *Hind*III-digested SV40 DNA; lanes 2 to 4 and 6 to 8 contain *Hind*III-digested plasmid DNA from tetracycline-resistant bacteria. Colonies analyzed in lanes 2 to 4 and 6 to 8 were shown to contain SV40 DNA by colony hybridization before gel analysis.

and -C SV40 fragments, was digested with *Hind*III, religated to itself, and transfected into HB101 cells, and plasmid DNA from the tetracycline-sensitive colonies was analyzed by gel electrophoresis. Six colonies containing the *Hind*III-B fragment were detected; again, no colonies containing the *Hind*III-C fragment were found.

Due to the continued inability to isolate tetracycline-sensitive colonies containing the *Hind*III-C insert, we hypothesized that insertion of the SV40 *Hind*III-C fragment into pACYC184 did not destroy the tetracycline resistance of the plasmid. To test this hypothesis, we screened tetracycline-resistant colonies from previous transfection experiments by colony hybridization with ³²P-labeled SV40 DNA. Twelve colonies hybridized to the SV40 probe. Analysis of the plasmid DNA from these colonies revealed *Hind*III-C fragments in all 12 colonies (Fig. 1). The orientation of the *Hind*III-C fragments relative to the tetracycline resistance gene was determined by restriction enzyme analysis. The physical maps of two of these recombinant plasmids (Fig. 2) show that pSV108 contained the SV40 *Hind*III-C fragment oriented with the SV40 early promoters proximal to the tetracycline resistance gene, whereas in pSV111 the C fragment was oriented with the SV40 late promoters proximal to the tetracycline resistance gene.

Because both orientations of the C fragment were found in tetracycline-resistant colonies, we

also determined the relative orientations of the B and C fragments in pSV79, which contained both fragments and was tetracycline sensitive. In pSV79, the B fragment was located between the tetracycline resistance gene and the C fragment (Fig. 2). Therefore, the SV40 promoters were separated from the tetracycline resistance gene by a distance equivalent to the insertion of the B fragment.

We investigated in more detail the degree of tetracycline resistance of the two *Hind*III-C recombinants (pSV108 and pSV111), along with the *Hind*III-B and -C recombinant pSV79 and a *Hind*III-B recombinant plasmid, pSV99. The degree of tetracycline resistance was determined by constructing growth curves for these recombinant plasmids grown in the presence of various amounts of tetracycline by the procedure of Tait et al. (14). HB101 cells and pACYC184 were used as controls in this study. The growth of pSV79 and pSV99 was affected markedly by tetracycline concentrations of 1 or 2 µg/ml and resembled that of HB101 cells in the presence of the antibiotic (Fig. 3). Interestingly, insertion of the SV40 *Hind*III-B fragment did not convey a

FIG. 2. Physical maps of pACYC184, SV40, and the recombinant plasmids pSV79, pSV99, pSV108, and pSV111. Arrows indicate the location of antibiotic resistance genes. Cm, Chloramphenicol; Tc, tetracycline; ori, SV40 origin of replication. Black areas indicate cloned SV40 inserts.

FIG. 3. Growth curves of *E. coli* HB101 and HB101 cells containing the indicated recombinant plasmids in the presence of various amounts of tetracycline: (●) 0 μg/ml; (○) 1 μg/ml; (▲) 2 μg/ml; (△) 5 μg/ml; (◐) 10 μg/ml; (◑) 15 μg/ml; (⦿) 25 μg/ml; and (⊗) 30 μg/ml.

tetracycline-resistant phenotype, even though this fragment has been shown by Lebowitz and co-workers (8) to contain initiation sites for *in vitro* transcription of SV40 DNA I by *E. coli* RNA polymerase. Tetracycline concentrations of 5 μg/ml or greater totally inhibited the growth of these cells.

In contrast, pSV108 and pSV111 showed greater resistance to tetracycline. Concentrations up to 5 μg/ml had very little effect on the growth rate of pSV108, whereas total inhibition of growth was seen with concentrations of 10 μg/ml or greater. The growth of pSV111 resembled that of pSV108, except that at a tetracycline concentration of 5 μg/ml there was a noticeable

decrease in the growth rate. Although both pSV108 and pSV111 showed a more marked resistance to tetracycline than did pSV99, pSV79, or HB101, they were not as resistant as was normal pACYC184, which was affected only at concentrations of 25 μg/ml or greater. Therefore, although insertion of the SV40 *Hind*III-C fragment into pACYC184 allowed some expression of the tetracycline resistance gene, it did not restore the plasmid to the levels of tetracycline resistance expressed by the parent plasmid. The expression of the tetracycline resistance gene was not dependent on the orientation of the SV40 *Hind*III-C fragment, since both orientations allowed expression. It is hy-

pothesized that in pSV108 the early promoter sequences of SV40 are responsible for tetracycline resistance, whereas in pSV111 the late SV40 promoter sequences are responsible for resistance. The results presented in this paper, however, do not directly discern whether bacterial RNA polymerase binds to these SV40 promoter sequences.

Other investigators have reported that procaryotic promoters can direct expression of the tetracycline resistance gene in recombinant plasmids. Selzer et al. (12) identified the promoters of the rII genes of bacteriophage T4, and Bohlander and co-workers (2) identified the promoter of the mercury operon by replacing the tetracycline resistance gene promoters in plasmids with foreign DNA fragments and then looking for expression of the tetracycline resistance gene. In addition to procaryotic promoters, several groups reported similar results with promoters of eucaryotic virus DNAs. Mitsialis and co-workers (10) identified a promoter region in the long terminal repeat of avian tumor virus DNA that could direct the expression of the tetracycline resistance gene in *E. coli* plasmids. McKnight and Meagher (9) isolated DNA fragments from the small cauliflower mosaic virus that were also active as promoters in *E. coli* by replacing the promoters of the plasmid pBKH4. Based on our results and those from other laboratories, procaryotic antibiotic resistance genes, such as the tetracycline resistance gene, provide excellent tools for the isolation and identification of both procaryotic and eucaryotic promoter sequences. Workers in several laboratories have reported the development of specific plasmid vectors capable of detecting DNA fragments containing promoter sequences (1, 3, 15). To our knowledge, the work reported in this manuscript represents the first demonstration that SV40 promoter sequences can efficiently direct transcription of procaryotic genes. In addition, the ability to quantitate the extent of tetracycline resistance conveyed by insertion of a given promoter at a known site provides a system in which levels of promoter activity can easily be measured and compared.

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