JOURNAL OF BACTERIOLOGY, July 1995, p. 4179–4182 Vol. 177, No. 14
0021-9193/95/$04.00+0
Copyright 1995, American Society for Microbiology

Two *recA* Genes in *Myxococcus xanthus*

NAOKO NORIOKA,† MEI-YIN HSU, SUMIKO INOUYE, AND MASAYORI INOUYE*

Department of Biochemistry, Robert Wood Johnson Medical School, Piscataway, New Jersey 08854

Received 16 February 1995/Accepted 4 May 1995

Two *recA* genes, *recA*$_1$ and *recA*$_2$, in *Myxococcus xanthus* were cloned by using the *recA* gene of *Escherichia coli*, and their DNA sequences were determined. On the basis of deduced amino acid sequences, RecA1 and RecA2 have 67.0% identity to each other and 60.5 and 60.9% identities to *E. coli* RecA, respectively. Expression of *recA*$_2$ was detected in both vegetative and developmental cells by Northern blot (RNA) analysis, and a threefold induction was observed when cells were treated with nalidixic acid. Repeated attempts to isolate a *recA*$_2$ disruption mutant have failed, while a *recA*$_1$ disruption mutant was readily isolated. Both the *recA*$_1$ and *recA*$_2$ genes expressed in *E. coli* complement the UV sensitivity of an *E. coli recA* strain.

The RecA proteins are known to play key roles in genetic recombination, DNA repair, and the SOS response in prokaryotes (see reference 11 for a review). The *recA* genes have been cloned and sequenced from a large number of bacteria. RecA proteins encoded by these genes, consisting of approximately 350 amino acid residues, have 56 to 100% identity to the RecA protein from *Escherichia coli* (11). Among these amino acid sequences, more than 100 invariant or conserved residues are considered to be essential for RecA function.

Identification and isolation of *recA* gene from *Myxococcus xanthus*. The chromosomal DNA from *M. xanthus*, isolated as described previously (21), was digested individually with restriction enzymes *Eco*RI, *Bam*HI, *Pst*I, *Sal*I, *Xho*I, and *Sma*I. Following digestion, DNA was separated by 0.7% agarose gel electrophoresis, transferred to nitrocellulose filters, and used for Southern blot analysis under the less-stringent conditions (16). To identify the *recA* gene of *M. xanthus*, the 550-bp *Pst*I-*Eco*RI fragment from the *recA* gene of *E. coli* that corresponds to amino acids Leu-77 to Glu-259 of *E. coli* RecA (3, 13) was used as a probe. For each digestion, two bands of almost identical intensity hybridized with the probe (data not shown). The band sizes were >25 and 18.6 kb for *Eco*RI, >23 and 4.1 kb for *Bam*HI, 12.5 and 2.2 kb for *Pst*I, 11.1 and 3.8 kb for *Sal*I, 21 and 5.6 kb for *Xho*I, and 2.0 and 1.5 kb for *Sma*I. This result indicates that *M. xanthus* most likely contains two independent, unlinked genes that are homologous to the *E. coli recA* gene.

In order to clone these genes, the 4.1-kb *Bam*HI fragment from *M. xanthus* chromosomal DNA was first cloned into the unique *Bam*HI site of pBR322 and the clone was designated pMXrecA1. The restriction map for pMXrecA1 is shown in Fig. 1A, and the 1.5-kb *Sma*I fragment within the *Bam*HI fragment coincides with the 1.5-kb *Sma*I fragment detected by Southern blot analysis. When this 1.5-kb *Sma*I fragment was used to probe the same filter described above under stringent conditions for Southern blot analysis, only one band was detected for each lane: 18.6, 4.1, 12.5, 3.8, 5.6, and 1.5 kb for *Eco*RI, *Bam*HI, *Pst*I, *Sal*I, *Xho*I, and *Sma*I, respectively (data not shown).

In order to clone the second *recA* gene that hybridized with the 550-bp *E. coli recA* probe, an *M. xanthus* genomic cosmid library described previously (18) was screened with the same probe. Positive cosmids were further analyzed by restriction enzyme digestion and Southern blot hybridization. P828 containing 34 kb of *M. xanthus* chromosomal DNA was identified to carry the *recA*$_2$ gene. A 2.2-kb *Pst*I fragment was isolated from P828 and cloned into the unique *Pst*I site of pBR322, and the clone was designated pMXrecA2. Its restriction map and the location of the *recA* gene are shown in Fig. 1B.

Amino acid sequence similarities of RecA1 and RecA2 to *E. coli* RecA. Figure 2 shows amino acid sequence alignments of *M. xanthus* RecA1 and RecA2 with *E. coli* RecA. There are 207 (60.5%) and 218 (60.9%) residues that are identical to *E. coli* RecA in RecA1 and RecA2, respectively. The identities are spread almost throughout the entire sequences. However, if the internal sequences of 200 residues (residue 52 to 251 for RecA1 and residue 53 to 252 for RecA2) are compared with that of *E. coli* RecA (residue 51 to 250), the identities increase to 73.5 and 75.5% for RecA1 and RecA2, respectively.

Interestingly, the degree of identity between RecA1 and RecA2 is not significantly higher than that of their identities to *E. coli* RecA. Between the same internal sequences of 200 residues described above, the identity is 78%. When the entire sequences are compared, their identity is 67.0%. It should be noted that within the C-terminal sequence of 51 residues of *E.*

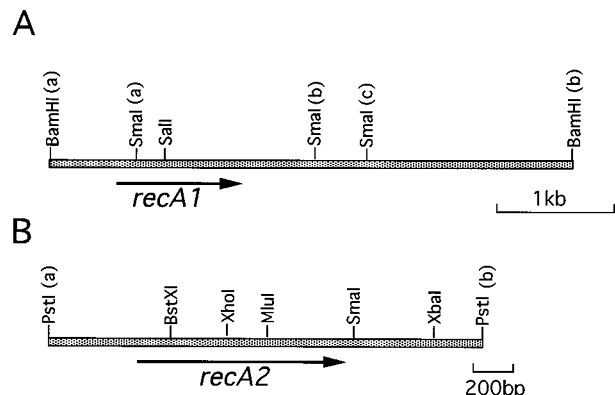

FIG. 1. Restriction maps of the DNA fragments encompassing the *M. xanthus recA*$_1$ and *recA*$_2$ genes. (A) 4.1-kb *Bam*HI fragment containing *recA*$_1$ and (B) 2.2-kb *Pst*I fragment containing *recA*$_2$. The arrows indicate the locations and orientations of the open reading frames.

* Corresponding author. Phone: (908) 235-4115. Fax: (908) 235-4783. Electronic mail address: inouye@umdnj.edu.

† Present address: Institute for Protein Research, Osaka University, 3-2 Yamadaoka, Suita, Osaka, 565 Japan.

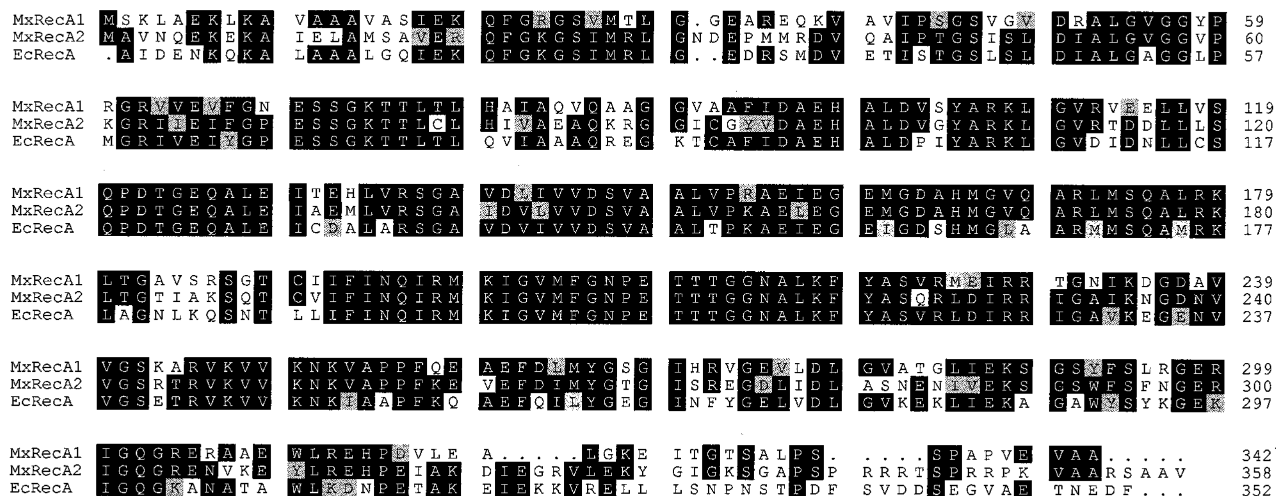

FIG. 2. Amino acid sequence alignment of $recA_1$ and $recA_2$ genes of *M. xanthus* (MxRecA1 and MxRecA2, respectively) and *recA* gene of *E. coli* (EcRecA). Identical residues are shown on a solid background and functionally similar residues are shown on a shaded background. The numbers on the right indicate residue numbers from the amino-terminal end. Note that the *E. coli* RecA protein starts with Ala, since the amino-terminal Met is known to be cleaved off.

coli RecA (residue 303 to 353), there are only 7 and 12 residues identical to those in the same region of RecA1 and RecA2, respectively. However, RecA1 and RecA2 contain 19 identical residues in this region. It is also interesting that this region of RecA2 is very basic (14 [R and K] residues versus 7 [D and E] residues) compared with that of *E. coli* RecA (6 [R and K] residues versus 12 D and E residues) or RecA1 (4 R and K residues versus 7 [D and E] residues). Generally, the C-terminal regions of RecA proteins are rich in acidic amino acid residues and have been proposed to play a role in interacting with DNA and in modulating a conformational charge required for RecA functions (11). While the C-terminal structure of the RecA2 protein is rather distinctive, at present the function is not known.

Expression and function of $recA_1$ and $recA_2$. The expression of the *recA* gene is known to be induced under SOS-responsive conditions such as UV irradiation or the addition of DNA synthesis inhibitors. To examine induction of the *recA* genes, exponentially growing cells in Casitone-yeast extract (CYE) were divided into two cultures in the presence or absence of 20 μg of nalidixic acid per ml and incubated at 30°C for 1 h. RNA was prepared by the method described previously (6) from a 40-ml culture. For Northern blot (RNA) hybridization, the RNA preparation from a 2-ml culture was applied to each lane. The amounts of RNA were examined by ethidium bromide staining of the gel. Northern blot hybridization was carried out with two synthetic oligonucleotides designed for the N-terminal regions of the $recA_1$ and $recA_2$ genes as described previously (8). Oligonucleotide 521 (5′ACCTTCTGTTCGCG TG3′) for $recA_1$ and oligonucleotide 519 (5′TCTCGCATCAT TGGCT3′) for $recA_2$ were complementary to DNA sequences from base 301 to 316 in the genes. These oligonucleotides were

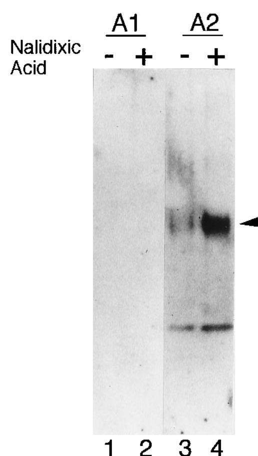

FIG. 3. Northern blot analysis of mRNAs for $recA_1$ and $recA_2$. RNA was isolated as described in the text. Lanes 1 and 3 contain RNA from cells not treated with nalidixic acid, and lanes 2 and 4 contain RNA from cells treated with 20 μg of nalidixic acid per ml. Hybridization was carried out with oligonucleotide 520 for $recA_1$ (A1) and with oligonucleotide 519 for $recA_2$ (A2). The arrow indicates the position of the $recA_2$ mRNA.

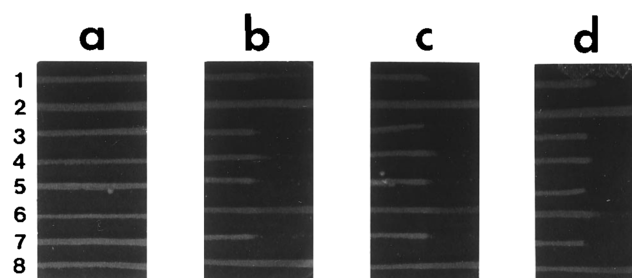

FIG. 4. UV sensitivity test of *E. coli recA* cells expressing *M. xanthus* $recA_1$ and $recA_2$. *E. coli* HMS174(DE3) harboring pET11a, pET11/recA1, or pET11/recA2 was grown in M9-CAA medium (M9 [8a] supplemented with 0.2% Casamino Acids) with or without 1 mM IPTG for 1 h. The cells were harvested by centrifugation, suspended in the same medium at a density of 3×10^9 cells per ml, and streaked onto M9-CAA agar plates. Half of the plates were covered with cardboard to prevent UV irradiation (a). The plates were then irradiated with a short-wavelength UV lamp (Mineralight model C81, Ultra-Violet Products, Inc.) at 30 cm from the plates for different lengths of time (0 [a], 40 [b], 60 [c], or 120 [d] s). After irradiation, the plates were incubated overnight at 37°C in the dark. Lane 1, *E. coli* SB221 (*recA*) harboring pUC18; lane 2, *E. coli* SB221 (*recA*) harboring pJC859 which contains the *E. coli recA* gene; lanes 3 and 4, *E. coli* HMS174(DE3) harboring pET11a; lanes 5 and 6, HMS174(DE3) with pET11/recA1; lanes 7 and 8, HMS174(DE3) with pET11/recA2. Cells were grown in M9-CAA medium without (lanes 3, 5, and 7) or with (lanes 4, 6, and 8) 1 mM IPTG.

TABLE 1. Bacterial strains, phages, and plasmids used in this study

Strain, phage, or plasmid	Genotype or description	Source or reference
Strains		
M. xanthus DZF1	*sglA*	5
E. coli		
JM105	*thi rpsL endA sbcB15 hspR4* Δ(*lac-proAB*)[F′*traD36 proAB lacI*^{q}ZΔM15]	20
HMS174(DE3)	F$^-$*recA hsdR Rif*^{r}(DE3)	17
SB221	*hsdR recA* Δ*trp leu thi lacY lpp* [F′ *lacI*^{q} *lacZY proA proB*]	9
Phages		
M13mp18	*lacZ* α fragment	20
M13mp19	*lacZ* α fragment	20
P1*clr100*Cm	Cm^r *clr100*	12
Plasmids		
pBR322	Tc^r Ap^r	10
pUC9	Ap^r *lacZ* α fragment	19
pP1EK	Ap^r P1*inc*	4
pUCkan1	Ap^r Km^r 2.5-kb *Hin*dIII-*Sal*I fragment of kanamycin-resistant gene from Tn*5*	
pUCkan5	Ap^r Km^r 2.5-kb *Bam*HI-*Sal*I fragment of kanamycin-resistant gene from Tn*5*	
pUC7km(Pst1$^-$)	Ap^r Km^r 1.3-kb *Hin*dIII-*Sma*I fragment of kanamycin-resistant gene from Tn*5; Pst*I in the gene was removed by site-directed mutagenesis	
pET11a	Ap^r T7 promoter	17
pJC859	*E. coli recA* gene	A. J. Clark
pUX	Ap^r pUC derivative	1
M. xanthus genomic cosmid library		
P595	12.5-kb *Pst*I fragment containing *recA*$_1$ gene	18
P828	34-kb *Pst*I fragment containing *recA*$_2$ gene	18
pMXrecA1	4.1-kb *Bam*HI fragment carrying *recA*$_1$ gene from *M. xanthus* chromosomal DNA ligated to *Bam*HI-digested pBR322 (Fig. 1A)	This work
pMXrecA2	2.2-kb *Pst*I fragment carrying *recA*$_2$ gene from P828 ligated to *Pst*I-digested pBR322 (Fig. 1B)	This work
pET11/recA1	After creation of *Nde*I at the initiation codon of the *recA*$_1$ gene by PCR with oligonucleotide 5′CTCATATGAGCAAGCTGGCG3′, 3.9-kb *Nde*I-*Bam*HI fragment was ligated in *Nde*I-*Bam*HI sites of pET11a	This work
pET11/recA2	By method for *recA*$_1$ with oligonucleotide 5′CTCATATGGCCGTGAATCAGG3′, 1.8-kb *Nde*I-*Bam*HI fragment was ligated in *Nde*I-*Bam*HI sites of pET11a	This work
pP1EK/ΔrecA1	1.0-kb *Bam*HI-*Sal*I fragment of pMXrecA1 was replaced with 2.5-kb kanamycin-resistant gene from pUCkan5. 1.0-kb *Pst*I-*Bam*HI fragment containing upstream of *recA*$_1$ gene from P595, 2.5-kb *Bam*HI-*Sal*I fragment of kanamycin-resistant gene, and 3.2-kb *Sal*I-*Bam*HI fragment from pMXrecA1 were ligated in *Pst*I-*Bam*HI sites of pP1EK	This work
pP1EK/ΔrecA2-1	1.3-kb *Sal*I fragment containing kanamycin-resistant gene was inserted in *Xho*I site of pMXrecA2 and then ligated to *Pst*I site of pP1EK	This work
pP1EK/ΔrecA2-2	Construct similar to pP1EK/ΔrecA2-1 carrying 2.1-kb extra fragment at the 5′-end arm	This work

phosphorylated at the 5′ end by T4 oligonucleotide kinase with [γ-^{32}P]ATP and were used as probes. As shown in Fig. 3, no bands were detected for *recA*$_1$ even after the nalidixic acid treatment. In contrast, *recA*$_2$ expression was significantly induced after this treatment (Fig. 3, lane 4). Interestingly, its expression was also clearly detected without nalidixic acid treatment. As determined by densitometry, nalidixic acid treatment increased *recA*$_2$ expression approximately threefold. Northern blot analysis was also carried out with RNA preparations from cells after 10 and 24 h of development. However, we were also unable to detect *recA*$_1$ mRNA, while *recA*$_2$ mRNA was detected (data not shown).

Next, we attempted to express both *recA*$_1$ and *recA*$_2$ in an *E. coli recA* strain and examined whether their gene products complemented the *E. coli* RecA function. To clone the *recA*$_1$ and *recA*$_2$ genes under the T7 promoter in pET11a, an *Nde*I site was created at the initiation codons of both genes by PCR, using the following primers: 5′CTCATATGAGCAAGCTGG CG3′ and 5′AGACCTCCACCACACG3′ (base 384 to 399 in the *recA*$_1$ DNA sequence) for the *recA*$_1$ gene and 5′CTCATA TGGCCGTGAATCAGG3′ and 5′TCCAGCGAGATGGAG3′ (base 338 to 352 in the *recA*$_2$ DNA sequence) for the *recA*$_2$ gene. The amplified fragments were cloned into the *Sma*I site of pUC9, and their DNA sequences were confirmed. In the case of *recA*$_1$, the 180-bp *Eco*RI (of pUC9)-*Sma*I, the 250-bp *Sma*I (a) (as shown in Fig. 1A)-*Sal*I, and the 3.5-kb *Sal*I-*Bam*HI fragments were cloned into the *Eco*RI-*Bam*HI sites of pUC9. The 3.9-kb *Nde*I-*Bam*HI fragment was isolated from the resulting plasmid and cloned into the *Nde*I-*Bam*HI sites of pET11a. The resulting plasmid was designated pET11/recA1. For *recA*$_2$, the 145-bp *Eco*RI (of pUC9)-*Bst*XI fragment and the 1.6-kb *Bst*XI (Fig. 1B)-*Bam*HI fragment from pUC19recA2 were first cloned into pUC9 and then recloned into pET11a in the same way as *recA*$_1$. The resulting plasmid was designated pET11/recA2. These constructs were transferred into *E. coli* HMS174(DE3) that carries the T7 polymerase gene. In the presence of 1 mM IPTG (isopropyl-β-D-thiogalactopyranoside), both RecA1 and RecA2 were expressed at more than 30% of the level of total cellular protein (data not shown).

As shown in Fig. 4, cells expressing either RecA1 (lane 6) or RecA2 (lane 8) after induction with IPTG clearly became more resistant to UV irradiation on an agar plate, indicating that both $recA_1$ and $recA_2$ genes encode functional RecA proteins. It should be noted, however, that cells with $recA_2$ were almost as resistant as *E. coli* $recA^+$ cells. In contrast, when $recA_1$ cells were UV irradiated for 120 s, only a few colonies appeared. This result suggests that RecA1 is less functional than RecA2 in *E. coli.*

Construction of $recA_1$ and $recA_2$ disruption strains of *M. xanthus.* Next, we attempted to isolate $recA_1$ and $recA_2$ mutant strains by homologous recombination with the plasmids described in Table 1. pP1EK/ΔrecA1 was transduced by P1 from *E. coli* to *M. xanthus* DZF1 by the method described previously (15). When 200 kanamycin-resistant transductants were screened with nick-translated pUC9 as a probe, 25 were negative. Subsequently, single colonies were isolated from eight independent transductants and analyzed by Southern blot hybridization with a nick-translated 12.5-kb *Pst*I fragment as a probe. Since it was known that the 2.5-kb kanamycin-resistant gene contained three *Pst*I sites, the chromosomal DNA from the $\Delta recA_1$ strain was confirmed to contain two positive bands, of 9.7 and 1.7 kb, instead of the 12.5-kb fragment from $recA_1$-containing DZF1 (data not shown). Therefore, the frequency of double crossover events in the case of $recA_1$ disruption was calculated to be 3%.

In contrast to the $\Delta recA_1$ results, the isolation of a $recA_2$ disruption mutant was unsuccessful. A total of 1,880 kanamycin-resistant colonies derived from two different plasmids in five independent experiments, including linearized pP1EK/ΔrecA2-2 transformants, were screened with nick-translated pUC9 as a probe. The mutants resulting from double crossover events were not identified. Since frequencies of double crossover events having the 3-kb left-side and the 0.97- to 1.75-kb right-side flanking fragments were 8 to 15% in the case of deletion mutants of *ops* and *tps* genes (2), the negative result with $recA_2$ most likely indicates that the $recA_2$ gene is essential for normal cell growth of *M. xanthus.*

The fact that RecA may be required during normal cell growth of *M. xanthus* is surprising, because a RecA requirement for cell growth has not been shown for any other bacteria to date. Further studies are necessary to elucidate the reason for the indispensability of RecA in *M. xanthus*. In this regard, it is interesting to examine whether RecA1, if expressed in *M. xanthus*, can complement the RecA2 function for cell growth.

Nucleotide sequence accession numbers. The nucleotide sequences of the $recA_1$ and $recA_2$ genes of *M. xanthus* were determined by the chain termination method, with single-stranded DNA used as templates (14) and were deposited in the GenBank/EMBL data library. The accession numbers are L40367 and L40368 for the $recA_1$ and $recA_2$ genes, respectively. The deduced amino acid sequences revealed open reading frames of 342 and 358 codons for $recA_1$ and $recA_2$, respectively, both of which harbor a putative ribosome binding sequence, GGAG, located 11 and 9 bases upstream from the initiation codons for $recA_1$ and $recA_2$, respectively. Both genes have inverted repeats 67 and 65 bases downstream of the termination codons for $recA_1$ and $recA_2$, respectively, which may serve as a transcription termination signal. Codon usages for both genes are typical for *M. xanthus*, and 88.6 and 91.9% of the codons use a G or C at the third position (7).

We thank J. Sun for her assistance and Linda Egger for her critical reading of the manuscript. We also thank T. Komano for the cosmid P828.

This work was supported by Public Health Service grant GM26843 from the National Institutes of Health.

REFERENCES

1. **Dhundale, A., M. Inouye, and S. Inouye.** 1988. A new species of multicopy single-stranded DNA from *Myxococcus xanthus* with conserved structural features. J. Biol. Chem. **263:**9055–9058.
2. **Furuichi, T., T. Komano, M. Inouye, and S. Inouye.** 1985. Functional complementation between the two homologous genes, *ops* and *tps*, during differentiation of *Myxococcus xanthus*. Mol. Gen. Genet. **199:**434–439.
3. **Horii, T., T. Ogawa, and H. Ogawa.** 1980. Organization of the recA gene of *Escherichia coli*. Proc. Natl. Acad. Sci. USA **77:**313–317.
4. **Hsu, M.-Y., S. Inouye, and M. Inouye.** 1989. Structure requirements of the RNA precursor for the biosynthesis of the branched RNA-linked multicopy single-stranded DNA of *Myxococcus xanthus*. J. Biol. Chem. **264:**6214–6219.
5. **Inouye, M., S. Inouye, and D. R. Zusman.** 1979. Gene expression during development of *Myxococcus xanthus*: pattern of protein synthesis. Dev. Biol. **68:**579–591.
6. **Inouye, S.** 1984. Identification of a development-specific promoter of *Myxococcus xanthus*. J. Mol. Biol. **174:**113–120.
7. **Inouye, S., M.-Y. Hsu, S. Eagle, and M. Inouye.** 1989. Reverse transcriptase associated with the biosynthesis of the branched RNA-linked msDNA in *Myxococcus xanthus*. Cell **56:**709–717.
8. **Inouye, S., and M. Inouye.** 1991. Site-directed mutagenesis using gapped-heteroduplex plasmid DNA, p. 71–81. *In* M. J. McPherson (ed.), Directed mutagenesis, a practical approach. Oxford University Press, New York.

8a. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

9. **Nakamura, K., Y. Masui, and M. Inouye.** 1982. Use of a lac promoter-operator fragment as a transcriptional control switch for expression of the constitutive *lpp* gene in *Escherichia coli*. J. Mol. Appl. Genet. **1:**289–299.
10. **Peden, K. W. C.** 1983. Revised sequence of the tetracycline-resistance gene of pBR322. Gene **22:**277–280.
11. **Roca, A. I., and M. M. Cox.** 1990. The RecA protein: structure and function. Crit. Rev. Biochem. Mol. Biol. **25:**415–456.
12. **Rosner, J. L.** 1972. Formation, induction, and curing of bacteriophage P1 lysogens. Virology **49:**679–689.
13. **Sancar, A., C. Strachelek, W. Konigsberg, and W. D. Rupp.** 1980. Sequences of the recA gene and protein. Proc. Natl. Acad. Sci. USA **77:**2611–2615.
14. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74:**5463–5467.
15. **Shimkets, L. J., R. E. Gill, and D. Kaiser.** 1983. Developmental cell interactions in *Myxococcus xanthus* and the *spo*C locus. Proc. Natl. Acad. Sci. USA **80:**1406–1410.
16. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98:**503–517.
17. **Studier, F. W., A. H. Rosenberg, J. J. Dunn, and J. W. Dubendorff.** 1990. Use of T7 RNA polymerase to direct expression of cloned genes. Methods Enzymol. **185:**60–89.
18. **Tojo, N., S. Inouye, and T. Komano.** 1993. Cloning and nucleotide sequence of the *Myxococcus xanthus lon* gene: indispensability of *lon* for vegetative growth. J. Bacteriol. **175:**2271–2277.
19. **Vieira, J., and J. Messing.** 1982. The pUC plasmids, an M13mp7-derived system for insertion mutagenesis and sequencing with synthetic universal primers. Gene **19:**259–268.
20. **Yanisch-Perron, C., J. Vieira, and J. Messing.** 1985. Improved M13 phage cloning vectors and host strains: nucleotide sequence of the M13mp18 and pUC19 vectors. Gene **33:**103–119.
21. **Yee, T., and M. Inouye.** 1981. Reexamination of the genome size of myxobacteria, including the use of a new method for genome size analysis. J. Bacteriol. **145:**1257–1265.