JOURNAL OF BACTERIOLOGY, Oct. 1988, p. 4939–4941
0021-9193/88/104939-03$02.00/0

Vol. 170, No. 10

NOTES

Tn*5*-Mediated Transposition of Plasmid DNA after Transduction to *Myxococcus xanthus*

JOHN S. DOWNARD

Department of Botany and Microbiology, University of Oklahoma, Norman, Oklahoma 73019

Received 14 March 1988/Accepted 11 July 1988

After coliphage P1-mediated transfer of Tn*5*-containing plasmid DNA from *Escherichia coli* to *Myxococcus xanthus*, transductants were identified which contained plasmid sequences integrated at many sites on the bacterial chromosome. The unaltered plasmid DNA sequences in these transductants were apparently flanked by intact Tn*5* or IS*50* sequences. These results suggest that Tn*5*-mediated transposition has occurred and provide a method for integrating plasmid DNA into the *M. xanthus* chromosome without the requirement for homologous recombination.

Myxococcus xanthus is a gram-negative gliding bacterium which forms multicellular fruiting bodies in response to nutritional downshift (10, 18). One aspect of this process is the regulation of gene expression in response to cell-cell interactions (8, 9, 11). To understand the mechanisms involved in this novel form of regulation, we have been investigating the *tps* and *ops* genes of *M. xanthus*. These highly related genes are developmentally regulated with distinct temporal patterns of expression (4, 6, 7). A routine aspect of these studies has been the construction of fusions of the *tps* or *ops* gene to the *lacZ* gene in *Escherichia coli*, followed by the transfer of the fusion genes to *M. xanthus* for phenotypic analysis. Coliphage P1-mediated transduction is used to transduce the plasmid DNA to *M. xanthus* (13), and since pBR322-derived origins of replication do not function in *M. xanthus*, homologous recombination is generally required to integrate the transferred DNA into the bacterial chromosome. At least 40 kilobase pairs (kbp) of DNA is required for encapsidation in P1 phage particles, and this DNA is transferred as a single linear molecule (17). Therefore, it appears that multimers of small plasmids, which are present in recombination proficient *E. coli* host strains, are the substrates for packaging. In this study I report another class of stable transductants which forms at low frequency without the requirement for homologous recombination. This class of transductants was observed when the transferred plasmid DNA included Tn*5*, and this element appeared to mediate transposition of the gene fusion DNA to the *M. xanthus* chromosome.

The construction of the plasmids used in this study has been described elsewhere (5). In general they consist of segments of *M. xanthus* DNA inserted into the *lacZ* fusion vector, pMLB1034 (14), with Tn*5* insertions in the vector ampicillin resistance gene. Tn*5* is a 5.7-kbp composite transposon consisting of terminal 1.5-kbp IS*50* elements, in inverted orientation, bracketing a region encoding kanamycin resistance (2). Each IS*50* is also a transposable element (3). The methods for the production of P1 lysates and transduction of the wild-type *M. xanthus* DZF1 have been described elsewhere (13). Transductants were identified by the Tn*5*-encoded kanamycin resistance (Kn^r) phenotype (50 μg/ml). Chromosomal DNA from *M. xanthus* transductants was isolated by the method of Avery and Kaiser (1). Plasmid DNA was ^{32}P-labeled by nick translation with a kit from Bethesda Research Laboratories, Inc., and Southern hybridization analysis and colony hybridization were performed essentially as described elsewhere (12).

After transfer of the plasmid pJDK10-51 (7), which contains a 1,036-base-pair (bp) fragment of *M. xanthus* DNA, 5 of the 40 Kn^r transductants were found to have the plasmid DNA sequences integrated outside the region of homology with the bacterial chromosome (Table 1). This conclusion was based on Southern hybridization analysis of chromosomal DNA from these strains. As has been observed previously (13), when plasmids with smaller segments of *M. xanthus* DNA were transduced to wild-type strain DZF1, fewer Kn^r transductants were observed (Table 1). However, when these transductants were tested by Southern hybridization analysis, the proportion of strains with plasmid DNA sequences integrated by nonhomologous recombination increased from three of six for a plasmid with a 700-bp insert of *M. xanthus* DNA to five of five for a plasmid with a 106-bp insert. A number of different gene fusion plasmids have now been transduced into *M. xanthus*, and in no case has homologous integration into the chromosome been observed when the *M. xanthus* insert was less than 400 bp. Attempts to transduce pBR322:Tn*5* DNA which has no homology to the *M. xanthus* chromosome were not successful.

The structure of plasmid DNA sequences integrated by nonhomologous recombination was investigated by Southern hybridization analysis. Chromosomal DNA isolated from the wild-type strain DZF1 and from three pJDK26 transductants was digested with *Pst*I for this analysis. The plasmid pJDK26 contains a 201-bp insert of *M. xanthus tps* gene DNA (5) and a Tn*5* insertion in the ampicillin resistance gene and has five *Pst*I restriction sites (Fig. 1). Three identical filters with the blotted DNA fragments were hybridized with different ^{32}P-labeled probes. Hybridization with a pJDK10 probe (7) (pMLB1034 plus a 1,036-bp *M. xanthus tps* gene DNA fragment) resulted in detection of a 15-kbp fragment in all the strains and a 7.2-kbp fragment in the three pJDK26 transductants (Fig. 2A). The 7.2-kbp fragment was the apparently unaltered fragment from pJDK26 which contains a *tps-lacZ* fusion, the *E. coli* plasmid origin of replica-

TABLE 1. Relative frequencies of integration into the *M. xanthus* chromosome by homologous and nonhomologous recombination for transduced plasmids

Plasmid[a]	Insert size[b] (bp)	No. of transductants[c]	No. of nonhomologous recombinants/ no. tested[d]
pJDK10-51	1,036	450	5/40
pJDK8-X	700	91	3/6
pJDK8-H	106	8	5/5
pBR322::Tn*5*	0	<1	

[a] Plasmid pJDK10-51 (13 kbp) has been described elsewhere (7). The plasmids pJDK8-X (12.7 kbp) and pJDK8-H (12.1 kbp) are deletion derivatives of pJDK8 (7) from which only *M. xanthus* DNA has been removed. The plasmid pBR322 has been described (3), and pBR322::Tn*5* is about 10 kbp in size. All plasmids have Tn*5* insertions in the ampicillin resistance genes of pBR322 or pMLB1034 (14).

[b] Amount of *M. xanthus* DNA in the indicated plasmids.

[c] Number of Kn^r transductants in strain DZF1 per 10^{10} P1 PFU.

[d] Number of Kn^r transductants with plasmid DNA integrated outside the region of homology with the *M. xanthus* chromosome compared with the total number of Kn^r transductants tested by Southern blot analysis.

tion, and the end of an IS*50* sequence (Fig. 1). This DNA fragment is also observed after *Pst*I digestion of pJDK26. The 15-kbp *M. xanthus* fragment contains the region of homology with the *M. xanthus* DNA in pJDK26, and its presence in the transductants indicates that integration did not occur by homologous recombination; integration of the recombinant plasmid into this chromosomal location would introduce *Pst*I sites within this region. Hybridization of the transductant DNA with the pMLB1034 vector probe (Fig. 2B, lanes 2 through 4) detected only the same 7.2-kbp plasmid DNA fragment shown in Fig. 2A. A pBR322::Tn*5*

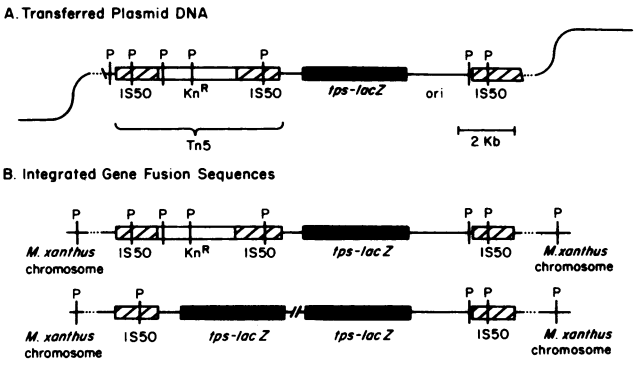

FIG. 1. Map of gene fusion plasmid DNA transferred during transduction and of the plasmid sequences found integrated into the *M. xanthus* chromosome. (A) An example of a transduced plasmid described in this study, pJDK26 (5), consists of a *tps-lacZ* fusion (▬), a pBR322 origin of replication (ori), and the transposon Tn*5* (bracketed, with component kanamycin resistance gene [▭] and IS*50* sequences [▨]). Apparently only multimers of this and the other plasmids tested in this report are large enough to be packaged and transferred to *M. xanthus* (13, 17). (B) The structures of the plasmid DNA sequences found in two typical transductants, which have this DNA integrated outside the *tps* gene region of the chromosome (nonhomologous recombination), are shown. For the upper chromosome, one copy of the fusion plasmid DNA was integrated between a copy of Tn*5* and the IS*50* from the adjoining copy of Tn*5*. For the lower chromosome, two plasmid copies were integrated between IS*50* sequences with a copy of Tn*5* (not shown) between them. The integrated DNA, in each case, was colinear with the transduced plasmid DNA shown in panel A. P, Restriction enzyme site *Pst*I.

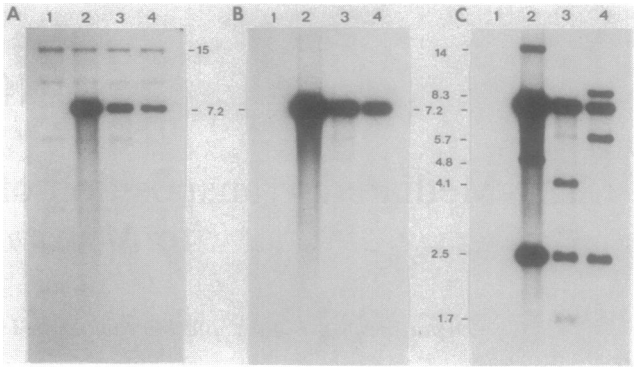

FIG. 2. Southern blot analysis of wild-type *M. xanthus* and pJDK26 transductants. Chromosomal DNA was isolated from *M. xanthus* DZF1 (lane 1) and from three pJDK26 transductants, JD13 (lane 2), JD91 (lane 3), and JD92 (lane 4). The DNA was digested with *Pst*I, fractionated by electrophoresis on a 0.7% agarose gel, and subjected to Southern hybridization analysis (12) with the following nick-translated probes: pJDK10 (7), a *tps-lacZ* fusion plasmid (A); pMLB1034, the *lacZ* fusion vector from which pJDK10 was constructed (B); pBR322::Tn*5* (C). The sizes of the restriction fragments observed by this analysis are shown in kilobase pairs.

probe with homology to the transposon and the vector DNA again hybridized with the 7.2-kbp band in all three transductants (Fig. 2C, lanes 2 through 4). This probe also hybridized to a 2.5-kbp fragment from Tn*5*, and two small additional Tn*5* fragments were observed in longer exposures of this autoradiogram (data not shown). In each of the transductants, two bands of variable size were observed. These bands contained Tn*5* and not vector sequences, since they were not observed with the pMLB1034 probe. These fragments appear to be junction fragments between Tn*5* sequences and variable segments of the *M. xanthus* chromosome.

Typical structures for the integrated DNA are indicated in Fig. 1B. The important features of the structures include the following: (i) the unaltered gene fusion and vector sequences are flanked by Tn*5* or IS*50* sequences, (ii) the flanking Tn*5* or IS*50* sequences are joined to chromosomal DNA sequences which do not have homology with the transduced plasmid, and (iii) the DNA is integrated at different sites in the *M. xanthus* chromosome for all the transductants which have been analyzed. Complete copies of IS50 (but not Tn*5*) were always present at the junctions between plasmid and chromosomal sequences. In some cases multiple copies of the plasmid DNA sequences were found integrated at the same site in the chromosome. An example of this situation is seen in strain JD13, in which the 7.2-kbp fragment was found in multiple copies as indicated by the increased hybridization signal (Fig. 2A, lane 2).

Most but not all Kn^r *M. xanthus* transductants contained gene fusion DNA sequences. Kn^r strains were tested by colony hybridization with the gene fusion vector probe to detect those transductants lacking the fusion sequences. Representative data from these tests of transductants with different gene fusion plasmids are presented in Table 2. Of a total of 73 independent Kn^r transductants tested, only 10 lacked gene fusion DNA sequences. In a few cases the transductants missing fusion gene sequences were tested by Southern hybridization analysis, and they all appeared to have simple insertions of Tn*5* in the *M. xanthus* chromosome (data not shown).

The simplest model to explain the observations reported in this study is that integration of plasmid DNA into the *M.*

TABLE 2. Frequency of kanamycin-resistant transductants with gene fusion DNA sequences

Plasmid[a]	Insert size[b] (bp)	No. of transductants with gene fusion DNA/no. tested[c]
pJDK26	201	35/40
pJDK26-D1	201	18/22
pJDK8-H	106	5/6
pJDK55-D3	417	5/5

[a] These plasmids have been described (5) with the exception of pJDK8-H, which is a deletion derivative of pJDK8 (7). All plasmids contain Tn*5* insertions and are close to 12 kbp in size.

[b] Amount of *M. xanthus* DNA in the indicated plasmids.

[c] Number of Kn^r transductants containing gene fusion plasmid DNA sequences, as determined by colony hybridization with a plasmid vector probe, compared with the total number of Kn^r transductants analyzed. The probe was the nick-translated vector, pMLB1034.

xanthus chromosome occurs by Tn*5*-mediated transposition. It appears that two flanking copies of Tn*5* (or IS*50*), on transferred multimeric plasmid DNA, act cooperatively to transpose the intervening DNA sequences. In the two examples shown in Fig. 1B, this model indicates that in the upper chromosome the transposed sequences extended from the outside end of Tn*5* to the inside end of IS*50* from the neighboring copy of Tn*5*, and in the lower sequence the transposed DNA extended from the inside end of one copy of IS*50* to the inside end of another copy with a complete copy of Tn*5* between fusion genes. Since IS*50* sequences are themselves transposable (3), it is not surprising that either IS*50* end might function in transposition. In all cases it appears that the integrated DNA is colinear with the structure predicted for transduced multimers of plasmid DNA.

The ability to integrate gene fusion plasmid DNA into the *M. xanthus* chromosome as described in this study has provided a simple method for analyzing *cis*-acting regulatory elements in this organism (5). Although this approach may be generally useful for such studies and for introducing foreign DNA into *M. xanthus*, the constraints imposed by the requirements for phage P1 packaging and prevention of homologous recombination will have to be considered when future experiments are designed. Another system has been developed recently for the integration of DNA segments into the *M. xanthus* chromosome. In this system the transduced DNA is integrated by site-specific recombination at the myxophage MX8 attachment site on the bacterial chromosome (15, 16). These methods for chromosomal integration of foreign DNA, without the requirement for homologous recombination, significantly increase the repertoire of tools available for the genetic analysis of *M. xanthus* development.

I thank Dave McCarthy and Doris Kupfer for reading the manuscript.

This work was supported by Public Health Service grant GM35462 from the National Institute of General Medical Sciences and a Biomedical Research Support grant from the National Institutes of Health.

LITERATURE CITED

1. **Avery, L., and D. Kaiser.** 1983. In situ transposon replacement and isolation of a spontaneous tandem genetic duplication. Mol. Gen. Genet. **191**:99–109.
2. **Berg, C. M., and D. E. Berg.** 1987. Uses of transposable elements and maps of known insertions, p. 1071–1109. *In* F. C. Neidhardt, J. L. Ingraham, B. Magasanik, K. B. Low, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
3. **Berg, D. E., L. Johnsrud, L. McDivitt, R. Ramabhadran, and B. J. Hirschel.** 1982. Inverted repeats of Tn*5* are transposable elements. Proc. Natl. Acad. Sci. USA **79**:2632–2635.
4. **Downard, J. S.** 1987. Identification of the RNA products of the *ops* gene of *Myxococcus xanthus* and mapping of the *ops* and *tps* RNAs. J. Bacteriol. **169**:1522–1528.
5. **Downard, J. S., S.-H. Kim, and K.-S. Kil.** 1988. Localization of the *cis*-acting regulatory DNA sequences of the *Myxococcus xanthus tps* and *ops* genes. J. Bacteriol. **170**:4931–4938.
6. **Downard, J. S., D. Kupfer, and D. R. Zusman.** 1984. Gene expression during development of *Myxococcus xanthus*: analysis of the genes for protein S. J. Mol. Biol. **175**:469–492.
7. **Downard, J. S., and D. R. Zusman.** 1985. Differential expression of protein S genes during *Myxococcus xanthus* development. J. Bacteriol. **161**:1146–1155.
8. **Gill, R. E., and M. G. Cull.** 1986. Control of developmental gene expression by cell-to-cell interactions in *Myxococcus xanthus*. J. Bacteriol. **168**:341–347.
9. **Kaiser, D.** 1986. Control of multicellular development: *Dictyostelium* and *Myxococcus*. Annu. Rev. Genet. **20**:539–566.
10. **Kaiser, D., C. Manoil, and M. Dworkin.** 1979. Myxobacteria: cell interactions, genetics, and development. Annu. Rev. Microbiol. **33**:595–639.
11. **Kuspa, A., L. Kroos, and D. Kaiser.** 1986. Intercellular signaling is required for developmental gene expression in *Myxococcus xanthus*. Dev. Biol. **117**:267–276.
12. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
13. **O'Connor, K., and D. R. Zusman.** 1983. Coliphage P1-mediated transduction of cloned DNA from *Escherichia coli* to *Myxococcus xanthus*: use for complementation and recombinational analyses. J. Bacteriol. **155**:317–329.
14. **Silhavy, T. J., M. L. Berman, and L. W. Enquist.** 1984. Experiments with gene fusions. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
15. **Stellwag, E., J. M. Fink, and J. Zissler.** 1985. Physical characterization of the genome of the *Myxococcus xanthus* bacteriophage MX-8. Mol. Gen. Genet. **199**:123–132.
16. **Stephens, K., and D. Kaiser.** 1987. Genetics of gliding motility in *Myxococcus xanthus*: molecular cloning of the *mgl* locus. Mol. Gen. Genet. **207**:256–266.
17. **Yarmolinsky, M. B.** 1977. Genetic and physical structure of bacteriophage P1 DNA, p. 721–732. *In* A. I. Bukhari, J. A. Shapiro, and S. L. Adhya (ed.), DNA insertion elements, plasmids, and episomes. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
18. **Zusman, D. R.** 1984. Cell-cell interactions and development in *Myxococcus xanthus*. Q. Rev. Biol. **59**:119–138.