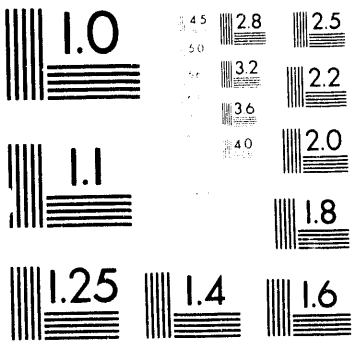
1.0
2.8
2.5
3.2
2.2
3.6
2.0
1.1
1.8
1.25
1.4
1.6

1 of 1

BNL-49412
BIO 4713

# Gene Expression in the *Dpn*I and *Dpn*II Restriction Enzyme Systems of *Streptococcus pneumoniae*

SANFORD A. LACKS, ALEXANDER G. SABELNIKOV, JAU-DER CHEN* and BILL GREENBERG

*Biology Department, Brookhaven National Laboratory, Upton, NY 11973, USA*

Proceedings of the 11th European Meeting on Genetic Transformation, DNA Transfer and Gene Expression in Microorganisms, Budapest, Hungary, August 23-27, 1992.

To be published by Intercept, Ltd., Newcastle upon Tyne, England.

Please address correspondence to Sanford A. Lacks (Tel. 516-282-3369)

MASTER

---

DISTRIBUTION OF THIS DOCUMENT IS UNLIMITED

* Present address: Department of Aquaculture, National Taiwan Ocean University, Keelung, Taiwan, Republic of China

**DISCLAIMER**

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

## Introduction

Although a number of bacterial species are naturally transformable, that is, their cells are able to take up external DNA in substantial amounts and integrate it into the chromosome without artificial manipulation of the cell surface, *Streptococcus pneumoniae*, the first species in which this phenomenon was detected, remains a prototype of such transformation. This is partly because these bacteria do not appear to use other forms of genetic exchange for transfer of chromosomal genes, such as conjugation and transduction, but rely solely on DNA-mediated transformation. Cells of *S. pneumoniae* also contain potent restriction endonucleases able to severely restrict DNA introduced during viral infection (Muckerman *et al.*, 1982). Therefore, it should be interesting to examine the effects of the restriction enzyme systems on transforming DNA. This is one purpose of the present work.

Our current understanding of the genetic basis of the complementary *Dpn*I and *Dpn*II restriction systems and of the biochemistry of their component enzymes will be briefly reviewed. The manner in which these enzymes impinge on the transfer of chromosomal genes and of plasmids will be examined in detail. It will be seen that far from acting against "foreign" DNA in general, the restriction systems seem to be designed to exclude only infecting viral DNA. The presence of complementary restriction systems in different cells of *S. pneumoniae* enhances their effectiveness in blocking viral infection and promoting species survival. This enhanced effectiveness requires the expression of alternative restriction systems. Therefore, the ability of the cells to transfer the restriction enzyme genes and to regulate their expression are important for survival of the species. The final part of this paper will present currently available information on this topic. In particular,

the localization of the restriction genes in cassettes, their transcription products, and the role of a possibly new class of ribosome binding sites will be examined in relation to the regulation of restriction gene expression.

**Genetics and biochemistry of the *Dpn*I and *Dpn*II restriction systems**

Strains of *S. pneumoniae* isolated from patients with pneumonia contain either the *Dpn*I or the *Dpn*II restriction system. *Dpn*I is an unusual restriction endonuclease in that it cleaves only the methylated sequence 5'-GmeATC in double-stranded DNA (Lacks and Greenberg, 1975; 1977). *Dpn*II is an ordinary restriction endonuclease, which, however, is complementary to *Dpn*I in that it cleaves unmethylated 5'-GATC sites (Lacks and Greenberg, 1977). Cells harboring the *Dpn*II system contain two enzymes that methylate these sites in double-stranded DNA: a major DNA methylase, DpnM, and a minor methylase, DpnA (de la Campa *et al.*, 1987).

The enzymes of the *Dpn*I and *Dpn*II systems are encoded by genetic cassettes that are alternatively located at a particular position in the chromosome of *S. pneumoniae* (Lacks *et al.*, 1986b). The presence of homologous DNA on either side of the restriction gene cassettes enables their exchange by genetic transformation. Such exchange was found to occur readily in the replacement of a defective *Dpn*I cassette by a *Dpn*II cassette (Muckerman *et al.*, 1982).

As indicated in Fig. 1, the *Dpn*I cassette encodes the 30-kDa endonuclease and another 18-kDa polypeptide of unknown function (de la Campa *et al.*, 1988). The *Dpn*II cassette encodes the two methyltransferases, DpnM and DpnA, that transfer methyl groups

from S-adenosylmethionine to the $N_6$ position of adenine in 5'-GATC sequences of DNA. DpnM, which corresponds to a polypeptide of 33 kDa (Mannarelli *et al.*, 1985) may exist either as a monomer (Cerritelli, White and Lacks, 1989) or a dimer (de la Campa *et al.*, 1987). DpnA, which is composed of a 31-kDa polypeptide is a dimer. The *Dpn*II endonuclease is a dimer of a 34-kDa polypeptide (de la Campa *et al.*, 1987).

Highly relevant to the selective effects of the restriction systems on different biological processes is the specificity of the enzymes for different forms of DNA. As illustrated in Fig. 2, neither *Dpn*I nor *Dpn*II can act on single-stranded DNA, whether methylated or not, or on hemimethylated DNA, where only one strand is methylated (Vovis and Lacks, 1977). For *Dpn*II only unmethylated, double-stranded DNA is a substrate, whereas for *Dpn*I, only DNA methylated in both strands is at all susceptible to cleavage.

**Restriction enzyme effects on viral infection and genetic exchange**

The *Dpn* systems very effectively inhibit infection by DNA-containing viruses that are grown on strains of complementary restriction phenotype. For example, bacteriophages grown on a *Dpn*II strain, and, therefore, containing methylated DNA, infect cells of a *Dpn*I strain at a frequency only one-millionth of that given by a *Dpn*II host strain (Muckerman *et al.*, 1982). *Dpn*II similarly restricts unmethylated bacteriophage DNA. The viral DNAs are presumably injected into the cell in a double-stranded form in which they are susceptible to cleavage by these endonucleases.

It has been proposed that the existence of complementary restriction systems contributes to the survival of the pneumococcal species by providing a surviving population

remnant in viral (bacteriophage) "epidemics" (Lacks *et al.*, 1987). If natural populations of *S. pneumoniae* contain mixtures of *Dpn*I and *Dpn*II type cells, then any outbreak of a viral epidemic, which would start with a single infective particle, would destroy only a portion of the bacterial population. For example, if the epidemic began with a virus grown on a *Dpn*I strain, only *Dpn*I cells in the population would be infected, and the *Dpn*II cells would survive. Eventually, once the epidemic passed, some of the survivors might be transformed to the *Dpn*I type, perhaps even by DNA released by lysis of the infected cells.

Although infecting viral DNA is highly restricted by the *Dpn* systems, transforming DNA appears to be very little affected. For chromosomal transformation, this is understandable because the processing of DNA during uptake into the cell and recombination with the host chromosome (indicated schematically in Fig. 3) renders the genetic information resistant to destruction by the *Dpn*I or *Dpn*II endonuclease irrespective of the methylation state of the donor DNA. Donor DNA is fragmented and converted to single strands during entry. Homologous donor strands interact with chromosomal DNA via a D-loop (as shown in Fig. 3) or by forming a triple-stranded structure as proposed earlier (Lacks, 1966), which by elimination of the homologous recipient strand is converted to a heteroduplex structure. Neither the single-strand segments that enter the cell nor the hemimethylated integration products would be susceptible to either restriction endonuclease. The absence of restriction of chromosomal transformation is shown for a $Mal^+$ marker in Table 1. However, it is somewhat surprising to find that plasmid transfer via the transformation pathway, indicated by the $Tc^r$ marker in Table 1, is only reduced by restriction to ~40%. In plasmid transfer, complementary single-strand segments of donor

DNA must anneal with each other to establish the replicon (Saunders and Guild, 1980), so the restoration of susceptible sites would be expected in the reconstituted plasmid. In the case of a methylated plasmid entering a *Dpn*I cell, however, it can be argued that due to the fragmentary nature of the complementing strands, much of the complements must be resynthesized by repair, and the newly synthesized DNA, being unmethylated, would leave a site hemimethylated and not susceptible to *Dpn*I (Lacks and Springhorn, 1984). This argument cannot be applied to an unmethylated plasmid entering a *Dpn*II cell, and the basis for escape from restriction in this case remained, until recently, a mystery.

**Role of the DpnA methylase in plasmid transfer**

The presence in the *Dpn*II system of two DNA methylases modifying the same sequence was also a mystery, particularly since a deletion mutation within the *dpnA* gene had no obvious effect on modification of cellular DNA or activity of the *Dpn*II restriction endonuclease. However, when this mystery was juxtaposed with the lack of restriction of plasmid transfer, the two mysteries canceled each other out. The deletion mutation, which removed a 389-bp *Dra*I segment from within the coding region of *dpnA*, was shown to render entering plasmids susceptible to restriction (Table 2). Furthermore, the extent of restriction was correlated with the number of 5'-GATC sites in the plasmid (Cerritelli, Springhorn and Lacks, 1989).

Discovery of the effect of a *dpnA* mutation on plasmid restriction led to an examination of the substrate structural specificity of the DpnA methylase and its comparison in this respect to DpnM (Cerritelli, Springhorn and Lacks, 1989). Table 3 shows that DpnM only acts on double-stranded DNA, whereas DpnA, although it acts weakly on double-

stranded DNA, methylates single-stranded DNA very well. Thus, DpnA methylates incoming plasmid strands prior to their interaction to reconstitute a double-stranded structure, thereby protecting unmethylated plasmid DNA from restriction by the *Dpn*II endonuclease. This analysis, by elucidating the mechanism for protection of plasmid DNA from restriction and by demonstrating the function of the additional methylase, resolved the two mysteries at once.

**Regulation of gene expression**

At least three cases have been encountered that require regulation of expression of the *Dpn*I and *Dpn*II system genes beyond the elementary start and stop signals for transcription and translation that are evident from their nucleotide sequences. **Case 1:** Introduction of the *Dpn*II system into a null cell. This is a highly efficient process (Muckerman, Springhorn and Lacks, 1982) that occurs during transformation of a defective *Dpn*I strain, such as strain Rx, which carries a frameshift mutation in the *dpnC* gene (Lacks, Springhorn and Cerritelli, 1991). This process requires expression of the methylase genes and methylation of the cellular DNA prior to expression of the *Dpn*II endonuclease gene. **Case 2:** Transitions between *Dpn*I and *Dpn*II cells. These transitions occur at a low frequency during transformation of a cell with an active restriction system by DNA from a cell of the opposite type (Lacks, unpublished data). Such transitions must require cessation of expression of the host system, blockage or dilution of its existing enzymes, and regulated expression of the new system. **Case 3:** Establishment of a *Dpn*I plasmid in a *Dpn*II cell. This mysterious process occurs frequently, but the *Dpn*I enzyme is not produced (Lacks, unpublished data).

The mechanism of repression here is not known. In addition to these special cases, the steady-state maintenance of the restriction systems might require regulated expression of their component genes. Possible clues to one or more of these regulatory processes may lie in the processing of their messenger RNAs or in the use of atypical ribosome binding sites for translation of some of their proteins.

## MESSENGER RNA IN THE *Dpn*II SYSTEM

Analysis of the *Dpn*II cassette sequence reveals a single consensus promoter and terminator that would give an RNA transcript of 2.7 kb, as indicated in Fig. 1. Similar analysis of the *Dpn*I cassette predicts a transcript of 1.4 kb. Preliminary results from Northern blotting of total RNA from *Dpn*I- and *Dpn*II-containing strains reveal mRNAs of these sizes (Fig. 4), but they also show that *Dpn*II strains contain smaller mRNAs corresponding to parts of the cassette. A particularly prominent band in strains in which multicopy plasmids carry the *Dpn*II cassette corresponds to an mRNA of 1.9 kb that hybridizes only to the *dpnA* and *dpnB* portions of the cassette. Two pairs of less prominent bands (only one pair of which is clearly evident in Fig. 4) could result from cleavage of the original transcript. In both cases putative RNase E recognition sites (Ehretsmann, Carpousis and Krisch, 1992) were found in the vicinity of the cleavage sites. These results are summarized schematically in the lower portion of Fig. 1. The possible significance of these mRNA molecules in the regulation of gene expression remains to be determined.

ATYPICAL RIBOSOME BINDING SITES

The translation start sites for the DpnM and DpnA proteins do not have any sequence similar to the one identified by Shine and Dalgarno (1975) as a ribosome binding site upstream from the starting codon. However, they were found to share a common sequence, which was proposed to be an atypical ribosome binding site (Mannarelli *et al.*, 1985; de la Campa *et al.*, 1987). This sequence is shown in Fig. 5 together with a similar sequence found upstream from the start codon for RepB (Lacks *et al.*, 1986a), which is a protein essential for replication of the plasmid pLS1 (de la Campa, del Solar and Espinosa, 1990). All three of these proteins that are presumably translated using the atypical ribosome binding site are expressed in both *S. pneumoniae* and *E. coli*, and protein expressed in *E. coli* was used to determine the translation start site. Of the 10 bases in the consensus sequence for the putative ribosome binding site, 9 show complementarity to a stretch of the 16S ribosomal RNA from *E. coli*, as indicated in Fig. 5. Furthermore, the complementary bases lie in an unpaired loop region of the 16S rRNA, and in the proposed three-dimensional structure of the 16S rRNA (Brimacombe *et al.*, 1988), this portion of the RNA molecule lies close to its 3'-terminus, which interacts with Shine-Dalgarno sequence (Shine and Dalgarno, 1975). Thus, the sequences in question exhibit a number of features consistent with a function of binding mRNA to the ribosome for the initiation of protein synthesis.

## Conclusions

The *Dpn*I and II restriction systems appear to be designed to block viral infection of *S.*

*pneumoniae* populations and to limit the damage in a bacterial population that does become infected. The substrate specificity of the enzymes is such as to destroy virus DNA but to leave transforming DNA unharmed. This is true for both chromosomal transformation and the transfer of plasmids. Most notably, the *Dpn*II system contains an enzyme, the DpnA methylase, whose sole function appears to allow transfer of unmethylated plasmids into the cells. The evolutionary burden of maintaining the *dpnA* gene for this purpose could be justified only by the significance of plasmid transfer for survival of the species. Both the design of the *Dpn* endonucleases and the existence of the DpnA methylase point up the great importance of genetic exchange for the survival of living organisms.

The control of *Dpn*I and *Dpn*II gene expression appears to occur on several levels as follows. **Level I:** Genetic recombination. The transfer of genetic cassettes from one cell to another by the pneumococcal transformation pathway can alter expression at the chromosomal level. **Level II:** Transcription of mRNA. In the *Dpn*II system a number of mRNAs smaller than the original transcript are found. They may result from RNA processing for the purpose of regulating gene expression. **Level III:** Translation of protein. The putative atypical ribosome binding sites associated with the *Dpn*II methylase genes may play a role in the relative expression of the methylase and endonuclease genes.

**Acknowledgements**

We are indebted to Dr. Adela G. de la Campa for pointing out the complementarity between the putative atypical ribosome binding sites and the sequence of 16S RNA.

Research was conducted at Brookhaven National Laboratory under the auspices of the U. S. Department of Energy Office of Health and Environmental Research. It was supported by U. S. Public Health Service grants AI14885 and GM29721 from the National Institutes of Health.

## References

BRIMACOMBE, R., ATMADJA, W. S., AND SCHULER, D. (1988). A detailed model of the three-dimensional structure of *Escherichia coli* 16 S ribosomal RNA *in situ* in the 30 S subunit. *Journal of Molecular Biology* **199**, 115-136.

CERRITELLI, S., SPRINGHORN, S. S., AND LACKS, S. A. (1989). DpnA, a methylase for single-strand DNA in the *Dpn*II restriction system, and its biological function. *Proceedings ofthe National Academy of Sciences USA* **86**, 9223-9227.

CERRITELLI, S., WHITE, S. W., AND LACKS, S. A. (1989). Crystallization of the DpnM methylase from the *Dpn*II restriction system of *Streptococcus pneumoniae*. *Journal of Molecular Biology* **208**, 841-842.

DE LA CAMPA, A. G., DEL SOLAR, G. H., AND ESPINOSA, M. (1990). Initiation of replication of plasmid pLS1. The initiator protein RepB acts on two distant DNA regions. *Journal of Molecular Biology* **213**, 247-262.

DE LA CAMPA, A. G., KALE, P., SPRINGHORN, S. S., AND LACKS, S. A. (1987). Proteins encoded by the *Dpn*II restriction gene cassette: two methylases and an endonuclease. *Journal of Molecular Biology* **196**, 457-469.

DE LA CAMPA, A. G., SPRINGHORN, S. S., KALE, P., AND LACKS, S. A. (1988). Proteins encoded by the *Dpn*I restriction gene cassette: Hyperproduction and characterization of the *Dpn*I endonuclease. *Journal of Biological Chemistry* **263**, 14696-14702.

EHRETSMANN, C. P., CARPOUSIS, A. J., AND KRISCH, H. M. (1992). Specificity of *Escherichia coli* endoribonuclease RNase E: in vivo and in vitro analysis of mutants in a bacteriophage T4 mRNA processing site. *Genes & Development* **6**, 149-159.

LACKS, S. (1966). Integration efficiency and genetic recombination in pneumococcal transformation. *Genetics* **53**, 207-235.

LACKS, S., AND GREENBERG, B. (1977). Complementary specificity of restriction endonucleases of *Diplococcus pneumoniae* with respect to DNA methylation. *Journal of Molecular Biology* **114**, 153-168.

LACKS, S., AND GREENBERG, B. (1975). A deoxyribonuclease of *Diplococcus pneumoniae* specific for methylated DNA. *Journal of Biological Chemistry* **250**, 4060-4066.

LACKS, S. A., LOPEZ, P., GREENBERG, B., AND ESPINOSA, M. (1986a). Identification and analysis of genes for tetracycline resistance and replication functions in the broad-host-range plasmid pLS1. *Journal of Molecular Biology* **192**, 753-765.

LACKS, S. A., MANNARELLI, B. M., SPRINGHORN, S. S., AND GREENBERG, B. (1986b). Genetic basis of the complementary DpnI and DpnII restriction systems of S. pneumoniae: An intercellular cassette mechanism. *Cell* **46**, 993-1000.

LACKS, S. A., MANNARELLI, B. M., SPRINGHORN, S., GREENBERG, B., AND DE LA CAMPA, A. G. (1987). Genetics of the complementary restriction systems DpnI and DpnII revealed by cloning and recombination in *Streptococcus pneumoniae*. In *Streptococcal Genetics*, J. J. Ferretti and R. Curtiss III (ed), pp. 31-41. American Society for Microbiology, Washington, DC.

LACKS, S. A., AND SPRINGHORN, S. S. (1984). Transfer of recombinant plasmids containing the gene for *Dpn*II DNA methylase into strains of *Streptococcus pneumoniae* that produce *Dpn*I or *Dpn*II restriction endonucleases. *Journal of Bacteriology* **158**, 905-909.

LACKS, S. A., SPRINGHORN, S. S., AND CERRITELLI, S. (1991). Restriction/modification systems of pneumococci: Why two methylases in the *Dpn*II system? In *Genetics and Molecular Biology of Streptococci, Lactococci, and Enterococci*, G. M. Dunny, P. P. Cleary, and L. L. McKay (ed), pp. 71-76. American Society for Microbiology, Washington, DC.

MANNARELLI, B. M., BALGANESH, T. S., GREENBERG, B., SPRINGHORN, S. S., AND LACKS, S. A. (1985). Nucleotide sequence of the *Dpn*II DNA methylase gene of *Streptococcus pneumoniae* and its relationship to the *dam* gene of *Escherichia coli*. *Proceedings of the National Academy of Sciences USA* **82**, 4468-4472.

MUCKERMAN, C. C., SPRINGHORN, S. S., GREENBERG, B., AND LACKS, S. A. (1982). Transformation of restriction endonuclease phenotype in *Streptococcus pneumoniae*. *Journal of Bacteriology* **152**, 183-190.

SAUNDERS, C. W., AND GUILD, W. R. (1980). Monomer plasmid DNA transforms *Streptococcus pneumoniae*. *Molecular and General Genetics* **181**, 57-62.

SHINE, J., AND DALGARNO, L. (1975). Determinant of cistron specificity in bacterial ribosomes. *Nature (London)* **254**, 34-38.

VOVIS, C. F. AND LACKS, S. (1977). Complementary action of restriction enzymes Endo R.DpnI and Endo R.DpnII on bacteriophage fl DNA. *Journal Molecular Biology* **115**, 525-538.

**Table 1.** Effect of *Dpn* restriction systems on plasmid transfer and chromosomal transformation*

| Recipient *Dpn* type | Donor DNA | Marker† | Transformants/ml with DNA from *Dpn*I strain (unmodified) | Transformants/ml with DNA from *Dpn*II strain (modified) | Restriction effect |
|---|---|---|---|---|---|
| I | pMV158 | $Tc^r$ | 7,400 | 2,700 | 0.36 |
| I | pLS70 | $Mal^+$ | 1,600,000 | 2,200,000 | 1.4 |
| II | pMV158 | $Tc^r$ | 6,600 | 15,000 | 0.44 |
| II | pLS70 | $Mal^+$ | 7,000,000 | 9,900,000 | 0.71 |

* Data from Lacks and Springhorn, 1984.

† $Tc^r$ measures plasmid transfer; $Mal^+$ measures chromosomal transformation.

**Table 2.** Effect of a *dpnA* mutation on restriction of plasmid transfer*

| Recipient *Dpn*II genotype | Donor DNA | Marker | Transformants/ml with DNA from *Dpn*I strain (unmodified) | *Dpn*II strain (modified) | Restriction effect |
|---|---|---|---|---|---|
| Null | pMV158 | $Tc^r$ | 2,800 | 2,600 | 1.08 |
| | chromosome | $Str^r$ | 90,000 | 100,000 | 0.90 |
| Wild | pMV158 | $Tc^r$ | 4,900 | 10,000 | 0.49 |
| | chromosome | $Str^r$ | 250,000 | 260,000 | 0.96 |
| *dpnA* | pMV158 | $Tc^r$ | 290 | 35,000 | 0.0083 |
| | chromosome | $Str^r$ | 320,000 | 760,000 | 0.42 |

* Data from Cerritelli, Springhorn and Lacks, 1989.

**Table 3.** Methylation of single-stranded and double-stranded DNA by DpnM and DpnA*

| DNA substrate | Methyl groups transferred to DNA† | |
|---|---|---|
| | DpnM | DpnA |
| Thymus DNA (double-stranded) | 320 | 97 |
| M13MP8 DNA (single-stranded) | <3 | 870 |

* Data from Cerritelli, Springhorn and Lacks, 1989.

† Picomoles per hour per microgram of enzyme.

## Figure legends

**Fig. 1.** *Dpn*I and *Dpn*II restriction gene cassettes and their products. *Upper part:* Chromosomes in *Dpn*I and *Dpn*II cells are indicated as lines with the heavy part of the line corresponding to the cassette. Open boxes represent coding regions of open reading frames. Arrows represent putative transcripts from consensus promoters (P) to terminators (T). Translation start sites are indicated on transcripts as closed circles (Shine-Dalgarno sites) and open circles (putative atypical ribosome binding sites). *Lower part:* Schematic representation of observed *Dpn*II system mRNAs; placement indicates extent and line width indicates abundance.

**Fig. 2.** Enzymatic properties of the restriction enzymes from *S. pneumoniae*. Vertical arrows indicate position of cleavage.

**Fig. 3.** Processing of DNA during the transformation of *S. pneumoniae*. Schematic depiction of stages in the entry of external DNA (heavy line) and its recombination with chromosomal DNA (fine line) within the cell of *S. pneumoniae* (oval structure with bulbous pores).

**Fig. 4.** Messenger RNAs of the *Dpn*I and *Dpn*II systems. Northern blot of total RNA from *S. pneumoniae* cells containing a *Dpn*I plasmid (pI), a *Dpn*II plasmid (pII), or a *Dpn*II cassette only in the chromosome (cII), hybridized with probes containing nick-translated, $^{32}$P-labeled DNA from the indicated *dpn* genes. Inferred sizes and compositions of radioactive bands are shown.

**Fig. 5.** Atypical prokaryotic ribosome binding sites. DNA sequences upstream of the ATG start codons for three proteins are shown. The *E. coli* 16S RNA is shown from nucleotide position 680 to 700.

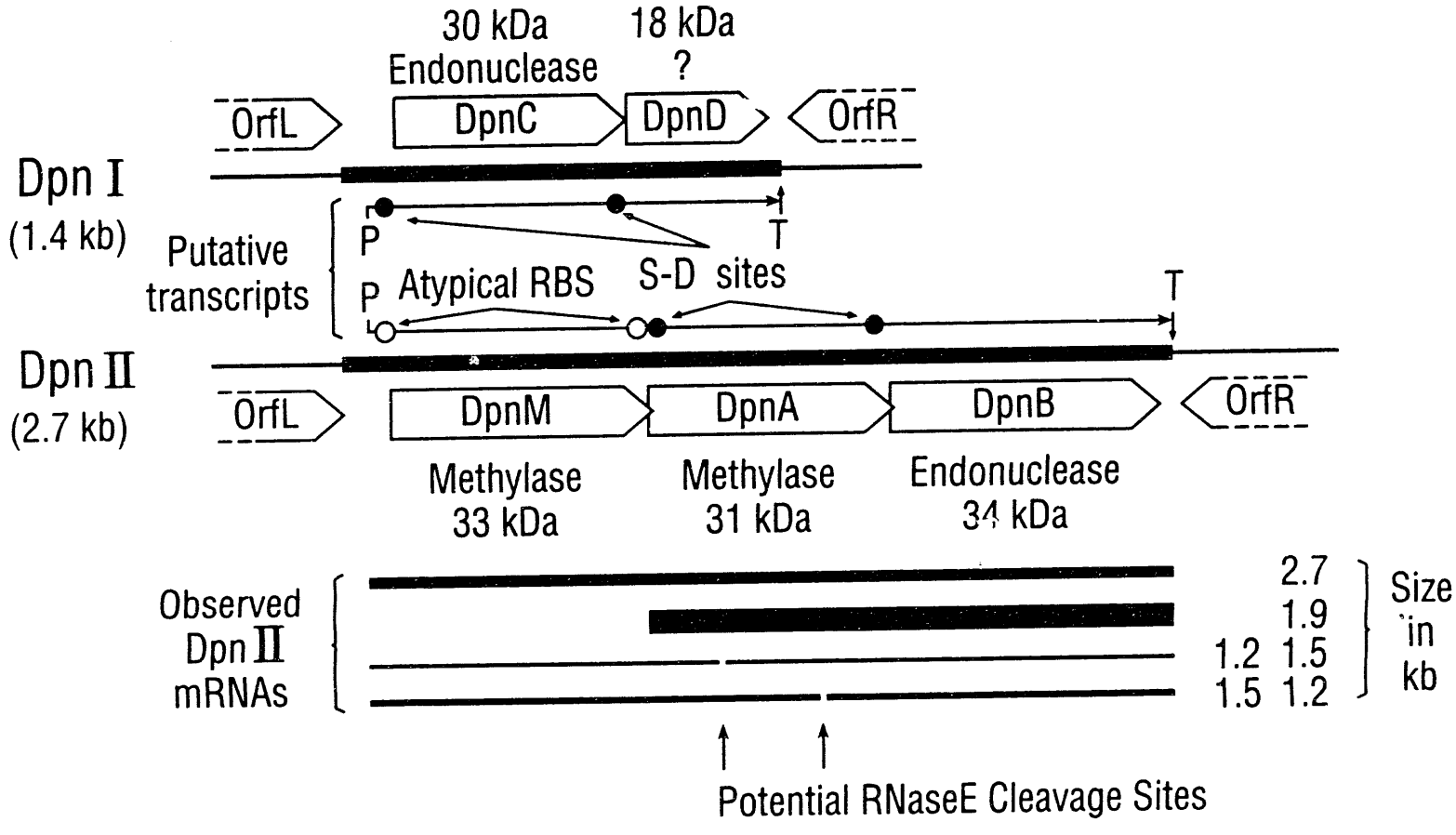
30 kDa
Endonuclease
18 kDa
?
OrfL
DpnC
DpnD
OrfR
Dpn I
(1.4 kb)
Putative
transcripts
P
T
P
Atypical RBS
S-D sites
T
Dpn II
(2.7 kb)
OrfL
DpnM
DpnA
DpnB
OrfR
Methylase
33 kDa
Methylase
31 kDa
Endonuclease
34 kDa
Observed
Dpn II
mRNAs
2.7
1.9
1.2
1.5
1.5
1.2
Size
in
kb
Potential RNaseE Cleavage Sites

Fig. 1

*Dpn*I endonuclease:

Cleaves
```
      me↓
5'...G A T C...3'
3'...C T A G...5'
      ↑me
```

*Dpn*II endonuclease:

Cleaves
```
      ↓
5'...G A T C...3'
3'...C T A G...5'
      ↑
```

*Dpn*II methylase:

Methylates
```
5'...G A T C...3'
3'...C T A G...5'
```
to give
```
      me
5'...G A T C...3'
3'...C T A G...5'
      me
```

Fig. 2

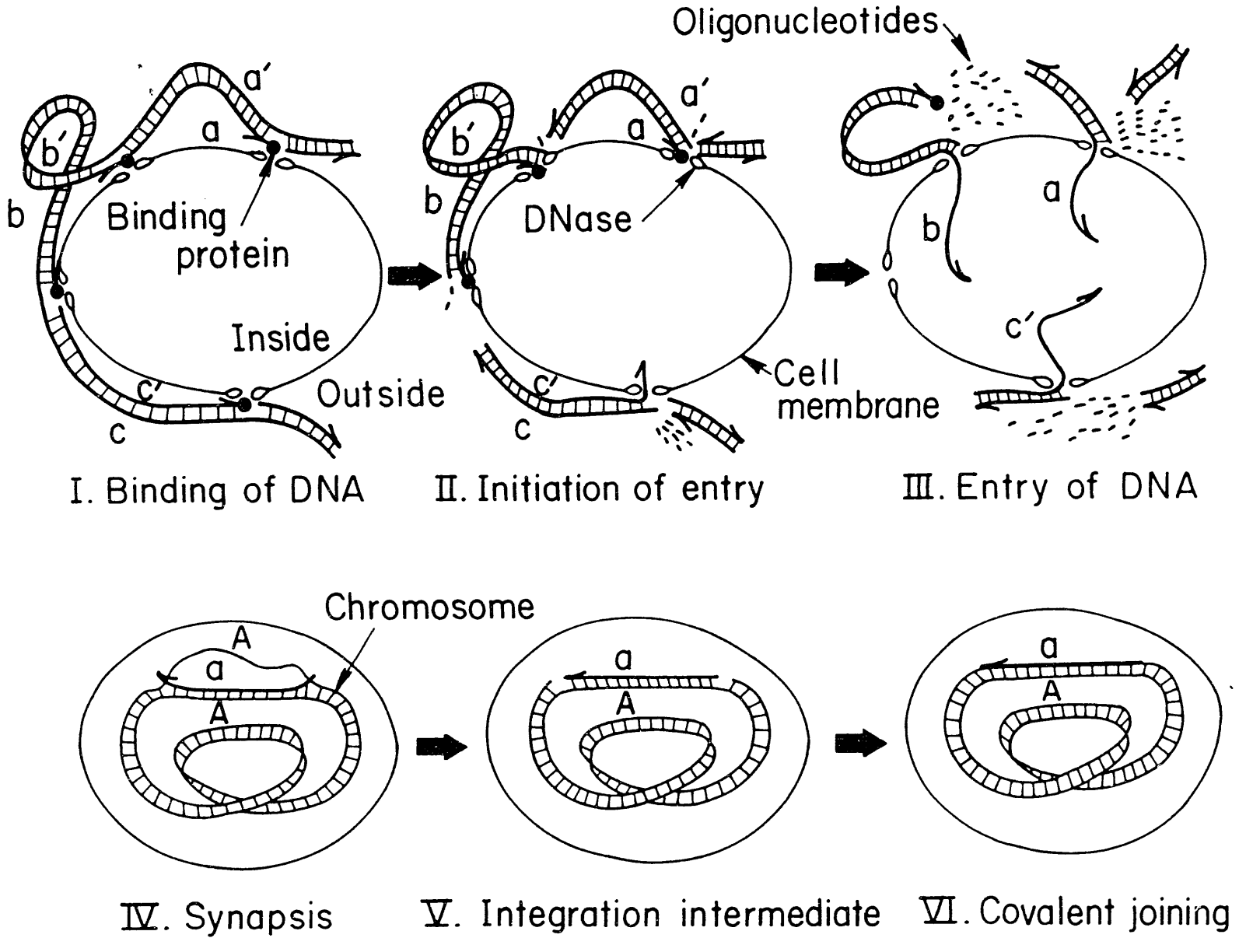
Oligonucleotides
a′
a
b′
b
Binding
protein
Inside
Outside
c′
c
DNase
Cell
membrane
I. Binding of DNA
II. Initiation of entry
III. Entry of DNA
Chromosome
A
a
IV. Synapsis
V. Integration intermediate
VI. Covalent joining

Fig. 3

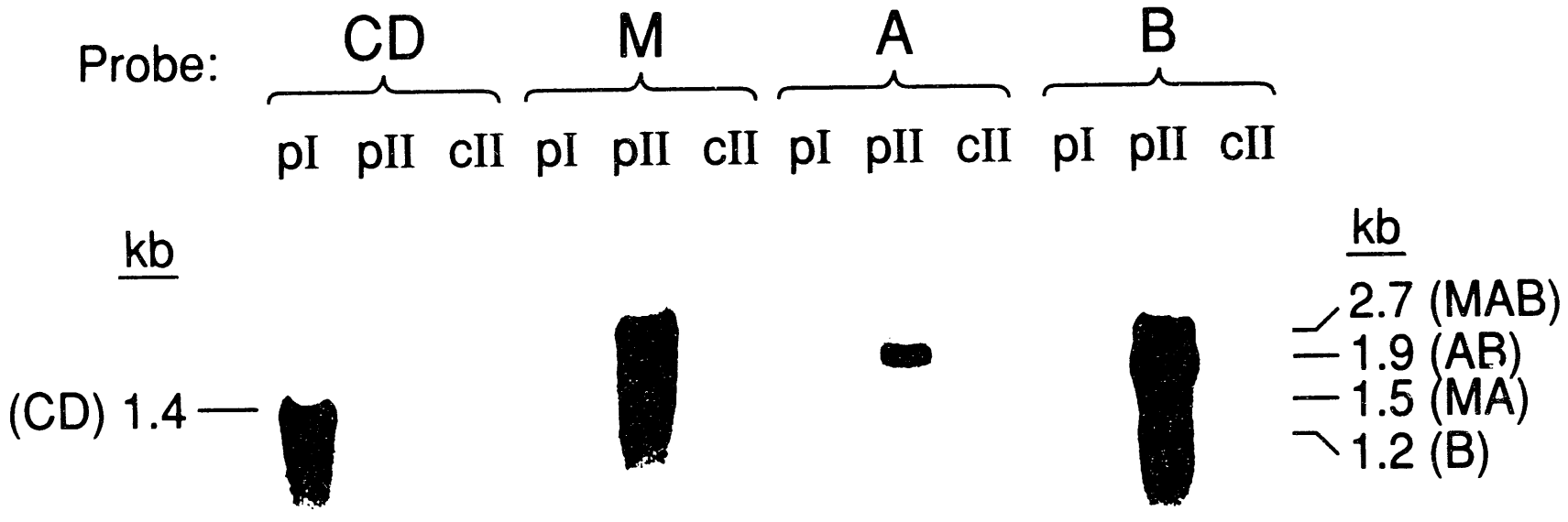
Probe:
CD
M
A
B
pI pII cII pI pII cII pI pII cII pI pII cII
kb
(CD) 1.4
kb
2.7 (MAB)
1.9 (AB)
1.5 (MA)
1.2 (B)

Fig. 4

| | | | | | | |
|---|---|---|---|---|---|---|
| **DpnM methylase:** | **...TGTTTTA** | **ATTTCT** | **ATGG** | **TATA** | **ATTAAAAGC** | **ATG** |
| **DpnA methylase:** | **...AGGAAAA** | **ATTTCT** | **GAAAT** | **TATA** | **GTCACAAATT** | **ATG** |
| **pLS1 RepB:** | **...GTCAAAC** | **ATTTCT** | **TGGG** | **TATA** | **TTATACTTT** | **ATG** |
| **Consensus sequence:** | | **ATTTCT** | **4-5 nt** | **TATA** | **9-10 nt** | **ATG** |
| **E. coli 16S rRNA:** | 700-**GCG** | **UAAAGU** | **GGCG** | **AUGU** | **UGGAC**-680 | |

Fig. 5

DATE
FILMED
12/13/93

END