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CHARACTERIZATION OF DNA ADENINE METHYLATION MUTANTS OF *Escherichia coli* K12

ALLEN BALE, MARC d'ALARCAO and M.G. MARINUS

Department of Pharmacology, University of Massachusetts Medical School, Worcester, MA 01605 (U.S.A.)

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Summary

The phenotypic traits of 7 independently isolated *dam* mutants of *Escherichia coli* have been examined. The mutant strains differ from the wildtype in the following respects: (1) decreased DNA adenine methylase activity in vivo and in vitro; (2) a 14—85-fold increase in spontaneous mutability; (3) decreased survival after ultraviolet irradiation; (4) a 10—21-fold increase in spontaneous induction of λ phage from lysogens; (5) a 3—17-fold increase in the level of recombination; and (6) inviability of double mutants containing dam^- and $recB^-$ or $recC^-$. Unmethylated fd phage chromosomes are able to replicate normally in dam^- mutants. A mutant strain in which the *dcm* gene is deleted is viable, showing that the *dcm* gene product is dispensible for growth.

Introduction

The *dam-3* mutant strain of *Escherichia coli* has low DNA adenine methylase activity in vivo and in vitro compared to wild-type [16]. In addition, the mutant strain has a pleiotropic phenotype and differs from wild-type in several respects which include hyper-recombination [15], increased sensitivity to alkylating agents and ultraviolet (UV) light; increased spontaneous mutability and increased spontaneous induction of phage λ from lysogens [18]. That the pleiotropic phenotype is due specifically to the *dam-3* mutation, however, is unclear since (i) this mutation was induced with *N*-methyl-*N*-nitro-*N*-nitrosoguanidine (NG), a mutagen known to produce multiple closely linked mutations [5] and (ii) that no dam^+ revertants have been isolated and characterized. In this communication, we describe the properties of 7 independently isolated *dam* mutants.

Abbreviations: 6-meA, 6-methyladenine; 5-meC, 5-methylcytosine; NG, *N*-methyl-*N*-nitro-*N*-nitrosoguanidine.

Materials and methods

Bacterial strains and bacteriophages. All the *dam* mutations were transferred into AB2847 [20] and its derivatives by P1 transduction using selection for AroB$^+$. The genotypes of some of the strains used in this work are given in Table 1. A modified genetic map of *E. coli* is shown in Fig. 1. Phages P1*vir* and λ*vir* were obtained from E.A. Adelberg, phage λ$^+$ from S. Champe; phage fd from S. Hattman.

Media. The minimal medium was that of Davis and Mingioli [3]. K medium is minimal medium supplemented with 1% decolorized Difco casamino acids. Complete medium is Difco brain heart broth (20 g/l) solidified, when required, with 1.6% Difco agar. L broth is that described by Luria and Burrous [13]. λ agar consists of 10 g tryptone, 5 g yeast extract, 5 g NaCl, 8.5 g agar and water to 1 l.

Genetic procedures. The methods for conjugation, transduction and selection of recombinants have been described elsewhere [14].

Isolation of dam mutants. The isolation of *dam-1*, *dam-2* and *dam-3* mutant strains by NG induction has been described elsewhere [16]. The strain bearing *dam-4* was isolated as a "hyper-rec" mutant by induction with ethyl methanesulfonate [9]. The *dam-5*, *dam-6* and *dam-7* mutant strains were isolated as follows. Strain W3110 *trpS10330* was mutagenized with NG and Trp$^+$ revertants selected which were unable to grow at 43°C and which had reduced amounts of 6-meA in DNA. The mutations in *dam* were transduced into strain AB2847 by selection for AroB$^+$. These transductants were no longer temperature sensitive for growth but still had reduced amounts of 6-methyladenine (6-meA) in DNA.

Isolation of an F-prime dam-3 episome and complementation tests. Strain Hfr GM42 (*dam-3*) was mated with JC1553 (*recA*) and Mal$^+$ clones were selected. One of these clones was shown to carry an F-prime element by (1) ability to transfer *argG*, *aroB*, *dam-3* and *mal* at high frequency; (2) could be cured by growth in broth, and (3) was phenotypically sensitive to streptomycin. This clone was designated F-*dam-3*/JC1553. The F-*dam-3* element was transferred by conjugation to wild type and other *dam* mutants by selection for

TABLE 1

GENOTYPES OF *E. coli* K-12 STRAINS [a]

Strain	Sex	Genotype
AB2847	F$^+$	*mal-354 aroB351 tsx-354*
F-*dam-3*/JC1553	F′	F-*argG*$^+$ *dam-3 malA*$^+$/*argG6 metB1 his-1 leu-6 recA1 mtl-2 xyl-7 malA1 gal-6 lacY1* or *Z4 str-104 sup-48 rel-1?*
GM42	Hfr	*dam-3 his*$^-$
JC1553	F$^-$ *str-104*	*argG6 metB1 his-1 leu-6 recA1 mtl-2 xyl-7 malA1 gal-6 lacY1* or *Z4 sup-48 rel-1?*
NA12	F$^-$	Δ(*supD-dcm-flaI,II*) *gal*$^-$ *tsx*$^-$ *bfe*$^-$ *str*^{R} λ*C1857*
NA1	F$^-$	As NA12 but Δ(*SupD*)

[a] Genetic symbols are those of Bachman et al. [1] except for *strA* (=*rpsL*). All strains are λ$^-$, λ^S unless otherwise indicated. Strains NA1 and NA12 were obtained from H. Kondoh.

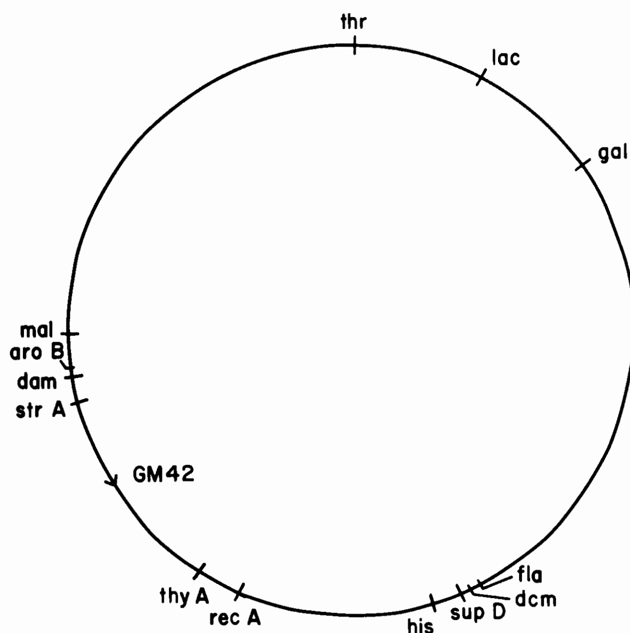

Fig. 1. Genetic map of *E. coli* K12 [1], showing pertinent genetic markers.

Mal$^+$. The partial diploids were grown in minimal medium, harvested and methyl-group incorporation into DNA determined as described in the following section.

In vitro assay of methyl-group incorporation into DNA. This procedure has been fully described elsewhere [14].

In vivo assay of methyl-group incorporation into DNA. This procedure has been described previously [14] except that RNA hydrolysis was accomplished by incubating in 0.3 M NaOH for 16 h.

Other methods. The mutation frequency was determined by inoculating 100 cells from each strain into 5 separate broths and allowing the cells to grow to saturation. Samples from each culture were plated on complete media containing streptomycin or spectinomycin or naladixic acid or rifampicin. After incubation for 2—3 days at 37°C, the plates were scored. Recombination was measured by determining the extent of conversion of *F-lac* heterogenotes to homogenotes as described previously [15]. The methods for propagation of phage fd and analysis of DNA on sucrose gradients have been described by Tseng and Marvin [22]. The efficiency of plating of phage λ, spontaneous induction of λ from lysogens, UV irradiations and sedimentation of DNA in alkaline sucrose gradients have been described elsewhere [18]. Pleiomorphism was qualitatively determined by examining crystal violet stained smears of bacterial cultures.

Results

(a) Adenine methylation of bacterial DNA

The mole percent 6-meA in DNA of *dam* and wild-type bacteria is shown in Table 2. The mutant strains fall into two classes: (1) those with no detect-

TABLE 2

CHARACTERIZATION OF *dam*$^+$ AND *dam*$^-$ MUTANT STRAINS

Genotype	Mole% 6-meA [a]	Mutation frequency [b]	Induction of phage λ [c]	E.O.P. λ.o [d]	Recombination index [e]	Filamentation
dam$^+$	2.0	1	1	1.0	1	—
dam-1	≤0.06	14	18	0.2	16	+
dam-2	0.53	55	17	0.1	17	+
dam-3	≤0.06	85	20	1.4	15	+
dam-4	≤0.06	54	18	1.7	9	+
dam-5	≤0.06	46	10	1.7	9	+
dam-6	≤0.06	65	11	0.1	10	+
dam-7	1.0	64	21	1.2	3	+

[a] The mole% 6-meA in vivo is the percent of adenines which are methylated.

[b] The relative frequency of resistance to naladixic acid and refampicin. The wild-type frequency was 2×10^{-8}.

[c] The relative spontaneous induction frequency of λ from lysogens. The frequency of free phage/bacteria for the wild type was 1.27×10^{-3}.

[d] The relative efficiency of plating of unmodified phage λ*vir*. The EOP for the wild type was 3.5×10^{-4}.

[e] The relative frequency fo Lac$^-$ segregants from F-*lac*$^+$/*lac*$^-$ heterogenotes. The wild-type frequency was 1%.

able amount of 6-meA in DNA and (2) those with detectable amounts of 6-meA in DNA. The limit of detection of 6-meA in the vivo assay is 0.06 mol% or about 3% of the wild-type level. 5 of the mutant strains have levels of 6-meA in DNA at or below this limit and 2 strains have 0.53 and 1.0 mol% 6-meA in DNA. All the mutant strains, except *dam-7*, have less than 1% residual DNA methylase activity in vitro relative to the wild-type (Fig. 2). The *dam-7* strain contained 8% DNA adenine methylase activity relative to wild-type (Fig. 2).

The in vitro results for the *dam-3* bacteria are in agreement with previously published data [16] but the in vivo results are not. The previous data suggested that the *dam-3* strain had 16% residual activity in vivo [16]. This discrepancy is due to the different methods used to solubilize RNA. Previously, ribonuclease was used to achieve this purpose whereas in this study alkali has been utilized. We suggest that ribonuclease treatment does not efficiently degrade RNA containing 6-methyladenosine and that some of this material contaminated the DNA fraction containing 6-methyldeoxyadenosine. Since the nucleic acids were subjected to subsequent acid hydrolysis it would not be possible to distinguish the 6-meA derived from RNA or DNA. Alkaline hydrolysis of RNA, by contrast, appears to overcome this contamination problem.

(b) Complementation tests

F-prime heterogenotes were constructed by transferring an F-prime element containing the *dam-3* mutation into each of the mutant strains. The DNA of these partial diploids was analysed by in vitro DNA methylation and the results are shown in Table 3. With the possible exception of the *dam-3/dam-7* diploid, no complementation of enzyme activity was observed, suggesting that the mutations are all located in the same cistron. The results from the *dam-3/dam-7* heterogenote are difficult to interpret because of the substantial residual

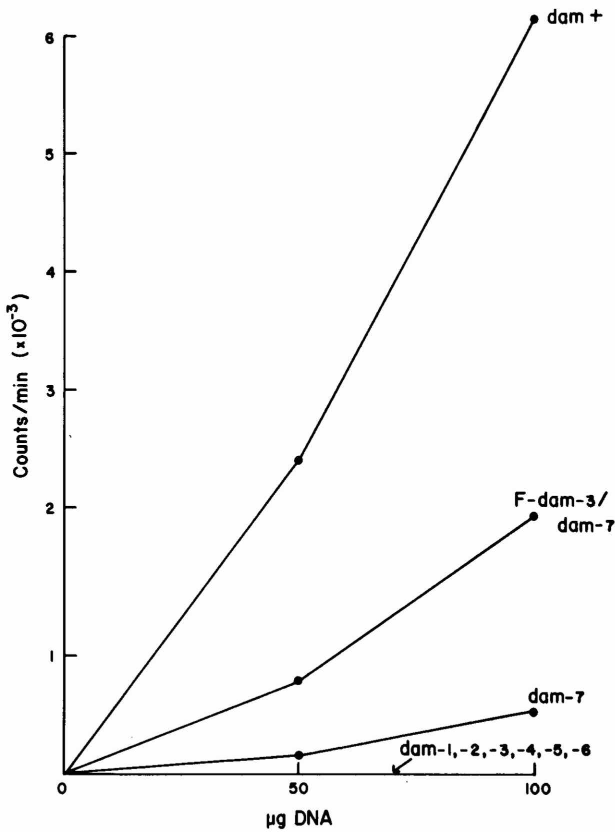

Fig. 2. Methyl group transfer into undermethylated DNA. Crude extracts were prepared from the wild type, *dam* mutants and an F-*dam-3*/*dam-7* partial diploid and assayed against undermethylated DNA obtained from a *dam-3* strain.

TABLE 3

METHYL-GROUP INCORPORATION INTO DNA

Nucleic acids were isolated from each of the trains and tested for ability to accept methyl groups into DNA in vitro using crude extract from the wild type.

Genotype	Counts/min/20 ml culture
dam$^+$	86
dam-3	4638
F-*dam*$^+$/*dam*$^+$	175
F-*dam-3*/*dam*$^+$	278
F-*dam-3*/*dam-1*	2944
F-*dam-3*/*dam-2*	3089
F-*dam-3*/*dam-3*	2399
F-*dam-3*/*dam-4*	2774
F-*dam-3*/*dam-5*	3322
F-*dam-3*/*dam-6*	4398
F-*dam-3*/*dam-7*	123
dam-7	2034

enzyme activity in the *dam-7* strain in vivo. No complementation of enzyme activity in mixtures of *dam-3* and *dam-7* crude extracts could be demonstrated. The mol% 6-meA in vivo in the diploid, however, was 1.9 compared to 1.1 in the *dam-7* strains alone, and undetectable in the *dam-3* strain. In crude extracts of the diploid, 32% of the normal level of DNA adenine methylase is present compared to 8% for the *dam-7* strain and less than 1% for the *dam-3* bacteria (Fig. 2). Whether complementation occurs in vivo or not is still to be resolved in this diploid.

(c) Survival of dam mutants after UV irradiation

The survival of the *dam* mutants and the wild-type after UV irradiation is shown in Fig. 3. All the mutant strains, except *dam-7*, have similar survival curves and these show an increased sensitivity to UV irradiation relative to wild-type. The *dam-7* strain is reproducibly more resistant than the other mutants although slightly more sensitive than the wild-type.

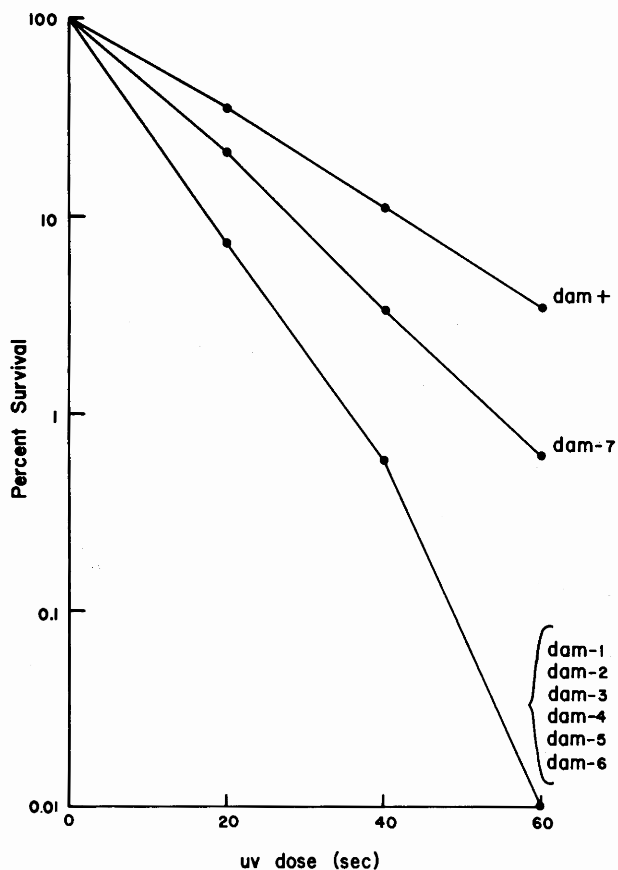

Fig. 3. Survival of *dam* mutants and the wild type after UV irradiation. Stationary phase cells at 5—10 × 10^6/ml were irradiated, diluted, plated on brian heart infusion agar and incubated in the dark for 48 h at 37°C.

(d) Other phenotypic traits of dam mutants

The results comparing the *dam* mutants with the wild-type for other phenotypic traits are shown in Table 2. In summary, the mutants (1) show a 14—85-fold increase in spontaneous mutability, (2) show an increased (10—21-fold) number of free λ phages in cultures of lysogens, (3) show a 3—17-fold increase in the level of recombination, (4) show pleiomorphism, and (5) are variable with respect to the plating efficiency of unmodified phage.

The DNA of the *dam* mutants has been analysed in alkaline sucrose gradients. In no case did the AB2847 *dam* derivatives show detectable single strand breaks. We have previously reported that a *dam-3* mutant (GM45), in an AB1157 background, did show single strand breaks in DNA. To reconcile this discrepancy 10 independently isolated transductants, from the original *dam-3* mutant were constructed, some of them in an AB1157 background. None of these clones showed any single strand breaks in DNA. It appears that the original *dam-3* strain contains a second mutation(s), causing single strand breaks, which is 9% linked to the *dam* gene.

Double mutants containing *dam-3* and *recA*, or *recB* or *recC* or *polA* or *lexA* are inviable [17,18]. Similarly, *dam-4* double mutants with *recB* or *recC* are inviable. The other *dam-4* combinations have not yet been tested.

(e) Growth of phage fd in dam-3 mutants

Bacteriophage fd contains a single stranded circular DNA chromosome [19] which contains 4 6-meA residues when propagated on wild-type strains [5,18]. If the phage is grown on a *dam-3* mutant, no 6-meA residues can be detected, viz., the number of 6-meA residues is much less than one per chromosome [18]. The growth curves of phage containing unmethylated DNA on dam^{+} and *dam-3* bacteria are indistinguishable (data now shown). We have also investigated the intracellular replicative forms of phage DNA in dam^{+} and *dam-3* bacteria, both early and late in the infective process. No difference could be detected in the molar amounts of RFI, RFII and progeny single strands between dam^{+} and *dam-3* infected bacteria. Unmethylated phage DNA chromosomes, therefore, appear to replicate normally.

(f) Characterization of a dcm deletion mutant

The *dcm* gene product is a DNA cytosine methylase which methylates cytosines in DNA at a level of 1.0 mol%. Mutant strains containing no detectable 5-methyl cytosine (5-meC) in DNA have been isolated [16]. Such strains appear to be normal with respect to mutagenesis, DNA replication, sensitivity to alkylating agents, etc. (Marinus, unpublished data) and it has been proposed that 5-meC is not required for viability [16].

Kondoh and Ozeki [8] and Kondoh [7] have recently isolated strains of *E. coli* with deletions in the *his-dcm-uvrC* region. Strain NA12 has a deletion extending from *supD* through *flaII*, and strain NA1 is deleted for *supD*. Strain NA12 has no DNA cytosine methylase activity in vivo or in vitro whereas strain NA1 has wild-type levels of this enzyme. These data indicate that the *dcm* gene has been deleted in strain NA12 and that this gene is closely linked to *supD* and *fla*, in agreement with previous genetic data [14]. We have confirmed the observations of Kondoh and Ozeki [8] that this strain has normal sensitivity to UV

light and also shown that it is normal for the parameters mentioned above. The data indicate that the *dcm* gene product is not required for viability.

Discussion

The aim of this investigation was to determine which of the phenotypic traits in the *dam*⁻ mutants are due to mutation in the *dam* gene. This could be done by comparing the properties of *dam*⁺ revertant to the original mutant strain. We have been unsuccessful, however, in isolating such revertants. The alternative was to examine the phenotypes of all the *dam*⁻ mutants and assume that those characteristics which are common to all mutants are due specifically to the *dam* mutation. It should be noted that, with the exception of *dam-4*, all the mutations were induced with NG. The *dam-4* mutation was induced with ethyl methanesulfonate. From the results comparing the properties of 7 *dam* mutants the following traits are associated with mutation in the *dam* gene: (1) decreased DNA adenine methylase activity in vivo and in vitro; (2) increased spontaneous mutability; (3) increased sensitivity to UV irradiation; (4) increased spontaneous induction of phage λ from lysogens; (5) an increase in level of genetic recombination; and (6) inviability of double mutants containing *dam*⁻ and *recB*⁻ or *recC*⁻.

The results in section (*a*) show that *dam* mutants have less than 3% residual in vivo adenine methylation. This result is now compatible with previous in vitro adenine methylation data [16] and is also compatible with the data obtained by Lacks and Greenberg [10]. The data above indicate that *dam-3* strains may be devoid of adenine methylation although Touissant [21] and Kushner (cited in [21]) suggest that some residual DNA adenine methylation may be present.

The phenotypic traits of *dam* mutants may allow the biological role of *dam* methylation to be elucidated. Models for such a role include protection of unmodified DNA from endonucleolytic cleavage [17]; strand discrimination in mismatch correction (Radman, Wagner and Meselson (cited in [4]); DNA synthesis [11,12]; the accommodation of plasmids [10] and in regulation of gene activity [6]. Since DNA methylation occurs at or near the replication fork [2,11] and that the phenotype of *dam* mutants resembles those of strains defective in DNA repair, we favor models invoking DNA repair occurring at or near the replication fork.

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References

1 Bachmann, B.J., K.B. Low and A.L. Taylor, Recalibrated linkage map of *Escherichia coli* K-12, Bacteriol. Rev., 40 (1976) 116–167.

2 Billen, D., Methylation of the bacterial chromosome: an event at the "replication point"? J. Mol. Biol. Biol., 31 (1968) 477–486.

3 Davis, B.D., and E.S. Mingioli, Mutants of *Escherichia coli* requiring methionine or vitamin B12, J. Bacteriol., 60 (1951) 17—28.

4 Glickman, B.W., P. van den Elsen and M. Radman, Induced mutagenesis in *dam*$^-$ mutants of *Escherichia coli:* a role for 6-methyladenine residues in mutation avoidance, Mol. Gen. Genet., 163 (1978) 307—312.

5 Guerola, N., J.L. Ingraham and E. Cerda-Olmedo, Induction of closely linked mutiple mutations by nitrosguanidine, Nature (London), New Biol., 230 (1971) 122—125.

6 Holliday, R., and J.E. Pugh, DNA modification mechanisms and gene activity during development, Science, 187 (1975) 226—232.

7 Kondoh, H., Isolation and characterization of nondefective transducing lambda bacteriophages carrying *fla* genes of *Escherichia coli* K-12, J. Bacteriol., 130 (1977) 736—745.

8 Kondoh, H., and H. Ozeki, Deletion and amber mutants of *fla* loci in *Escherichia coli* K-12, Genetics, 84 (1976) 403—421.

9 Konrad, E.B., Method for the isolation of *Escherichia coli* mutants with enhanced recombination between chromosomal duplications, J. Bacteriol., 130 (1977) 167—172.

10 Lacks, S., and B. Greenberg, Complementary specificity of restriction endonucleases of *Diplococcus pneumoniae* with respect to DNA methylation, J. Mol. Biol., 114 (1977) 153—168.

11 Lark, C., Studies on in vivo methylation of DNA in *Escherichia coli* 151$^-$, J. Mol. Biol., 31 (1968) 389—399.

12 Lark, C., Effect of the methionine analogues, ethionine and norleucine on DNA synthesis in *Escherichia coli* 15 T$^-$, J. Mol. Biol., 31 (1968) 401—414.

13 Luria, S.E., and J.W. Burrous, Hybridization between *Escherichia coli* and *Shigella,* J. Bacteriol., 74 (1957) 461—476.

14 Marinus, M.G., Location of DNA methylation genes on the *Escherichia coli* K-12 genetic map, Mol. Gen. Genet., 127 (1973) 47—55.

15 Marinus, M.G., and E.B. Konrad, Hyper-recombination in *dam* mutants of *Escherichia coli* K-12, Mol. Gen. Genet., 149 (1976) 273—277.

16 Marinus, M.G., and N.R. Morris, Isolation of DNA methylase mutants of *Escherichia coli* K-12, J. Bacteriol., 114 (1973) 1143—1150.

17 Marinus, M.G., and N.R. Morris, Biological function for 6-methyladenine residues in the DNA of *Escherichia coli* K-12, J. Mol. Biol., 85 (1974) 309—322.

18 Marinus, M.G., and N.R. Morris, Pleiotropic effects of a DNA adenine methylation mutation (*dam-3*) in *Escherichia coli* K-12, Mutation Res., 28 (1975) 15—26.

19 Marvin, D.A., and B. Hohn, Filamentous bacterial viruses, Bacteriol. Rev., 33 (1969) 172—209.

20 Pittard, J., and B.J. Wallace, Distribution and function of genes concerned with aromatic biosynthesis in *Escherichia coli,* J. Bacteriol., 91 (1966) 1494—1508.

21 Toussaint, A., The DNA modification of phage Mu-1 requires both a host and phage function, J. Virol., 23 (1977) 825-827.

22 Tseng, B.Y., and D.A. Marvin, Filamentous bacterial viruses, V. Asymmetric replication of fd duplex deoxyribonucleic acid, J. Bacteriol., 110 (1972) 371—383.

23 Wagner, R., and M. Meselson, Repair tracts is mismatched DNA heteroduplexes, Proc. Natl. Acad. Sci. (U.S.A.), 73 (1976) 4135—4139.

24 Witkin, E., Ultraviolet mutagenesis and inducible DNA repair in *Escherichia coli,* Bacteriol. Rev., 40 (1976) 869—907.