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Proteolytic Control of the Antirestriction Activity of Tn*21*, Tn*5053*, Tn*5045*, Tn*501*, and Tn*402* Non-Conjugative Transposons

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Abstract—Conjugative plasmids and conjugative transposons contain the genes, whose products specifically inhibit the type-I restriction-modification systems. Here, it is shown that non-conjugative transposons Tn*21*, Tn*5053*, Tn*5045*, Tn*501*, and Tn*402* partially inhibit the restriction activity of the type-I restriction-modification endonuclease EcoKI ($R_2M_2S_1$) into *Escherichia coli* K12 cells (phenomenon of restriction alleviation, RA). The antirestriction activity of the transposons is determined by the MerR and ArdD proteins. The antiresticton activity of transposons is absent in the *clpX* and *clpP E. coli* K12 mutants and is decreased in *recA*, *recBC*, and *dnaQ* (*mutD*) *E. coli* K12 mutants. The induction of the RA in response to the MerR and ArdD activities is consistent with the production of unmodified target sequences following DNA repair and DNA synthesis associated with recombination repair or replication errors. The RA effect is determined by the ClpXP-dependent degradation of the endonuclease activity R subunit of EcoKI ($R_2M_2S_1$).

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Keywords: antirestriction, type-I restriction-modification enzymes, non-conjugative transposon, Tn*21*, Tn*5053*, Tn*5045*, Tn*501*, Tn*402*, *MerR*, *ArdD*, protease ClpXP

INTRODUCTION

Conjugative plasmids and conjugative transposons contain genes *ardA* and *ardB*, which encode antirestriction proteins ArdA and ArdB that specifically inhibit the type-I restriction-modification enzymes [1–6]. Proteins of the ArdA subfamily simultaneously inhibit both restriction (endonuclease) and modification (methylase) activities of the enzymes [3–5], whereas proteins of the ArdB subfamily affect the restriction activity only [6, 7]. These proteins differ considerably from one another in both primary and tertiary structure. ArdA proteins, which consist of 165–170 amino acid residues, are characterized by significant negative charge (–25 to –30) and belong to the family of DNA-mimic proteins, i.e., their spatial structure is similar to that of the B form of double strand DNA [5]. ArdB proteins, which consist of 141–153 amino acid residues, are typically characterized by small negative charge (–1 to –6) and compact tetrahedron structure [7]. *ardA* and *ardB* genes help mobile elements overcome restriction barriers and, thus, provide efficient horizontal gene transfer between bacteria of different species and genera.

It has been shown that the full-length mercury non-conjutive transposon Tn*5053*, which contains two operons, the mercury-resistance operon *merRTPFADL* and the oppositely oriented operon *tniABQR*, decreased the restriction activity of the type-I EcoKI by approximately 200–400 times (the effect of restriction alleviation, RA) if introduced into *Escherichia coli* K12 within the pUC19 vector [8]. The new *ardD* gene, which is located inside *tniA* (encodes the TniA transposase), has been cloned. Moreover, the product of the *ardD* gene demonstrated antirestriction activity against EcoKI [8]. The antirestriction effect against EcoKI enzyme was formerly shown also for the *merR* gene, which was introduced into *E. coli* K12 within the pUC vector and encoded the repressor protein of the *mer* operon that determines mercury's resistance of bacteria [9].

In the present study, we estimated the antirestriction activity of non-conjugative transposons Tn*21*, Tn*5053*, Tn*5045*, Tn*501*, and Tn*402*, and examined the effect of the ClpXP protease and the *recA*, *recBC*, *dnaQ*, and *dam* genes on their antirestriction activity. It was shown that the antirestriction activity of transposons was absent in *E. coli* strains with mutation in *clpX* and *clpP* genes and considerably decreased in the strains mutant by the *recA*, *recBC*, and *dnaQ* genes.

EXPERIMENTAL

Bacterial Strains, Bacteriophages, and Plasmids

The bacterial strains and plasmids used in this study are described in Table 1.

Bacteriophage λ_{vir} was obtained from R. Devoret (France). Unmodified phages λ.0 and modified

Table 1. Bacterial strains and plasmids

Strain	Genotype	Source or reference
AB1157	F$^-$*thr-1, leu-6, pro*A2, *his-4, thi-1, argE3, lacY1, galK2, ara14, xyl-5, mtl-1, tsx-33, rpsL31, supE44*	GosNIIgenetika State Research Center
JM109	*recA1 endA1 gyrA96 thi supE44 relA1 hsdR17* Δ(*lac-proAB*) [*F'traD36 proAB lacI^{q}Z* Δ*M15*]	GosNIIgenetika State Research Center
TG-1	*thi relA supE44 hsdR17 hsdM* Δ(*lac-proAB*) *[F'traD36 proAB lacIqZ* Δ*M15*]	GosNIIgenetika State Research Center
JC7623	AB1157 *recB21 recC22 sbcB15*	GosNIIgenetika State Research Center
AB2463	AB1157 *recA13*	GosNIIgenetika State Research Center
NK113	AB1157 Δ*clpP::cat*	N.E. Murray, University of Edinburgh, Scotland
NK114	AB1157 Δ*clpX::kan*	N.E. Murray, University of Edinburgh, Scotland
VK0013	AB1157 *dam::kan*	This paper
VK0014	AB1157 *dnaQ* (*mutD*)::*kan*	This paper
BW25113	*lacI^{q}rmB3* Δ*lacX4787 hsdR5114* Δ*araBAD567*	Keio Collection
JW3350	BW25113 *dam::kan*	Keio Collection
JW0205	BW25113 *dnaQ744*(*del*)*::kan*	Keio Collection
pUB837	Tc^r Ap^r Sp^r Su^r Hg^r (pBR322::Tn*21*)	[10]
pVS982	Tc^r Ap^r Hg^r (pBR322::Tn*501*)	[10]
pKLH53.1	(pUC19 ::Tn*5053*)	[11]
pKLH402.1	(pUC19::Tn*402*)	[11]
pKLH45.1	pGEM-7Zf(–)::Tn*5045*	[12]
pTLORF5	pUC19 with 2300-bp fragment of the Tn*5053* transposon, which contains the *ardD* gene that was cloned by the KpnI–SalI sites	[8]
pKLH53.100	pUC19 with approximately 500-bp fragment of the Tn*5053* transposon, which contains the *merR* gene that was cloned by the SalI–HindIII sites	[9]
pAC-mer	merRTPFADL genes from the Tn*5053* transposon that were cloned by the ClaI–SalI sites	This paper
pardD-Tn21	pTZ57R with the cloned *ardD* gene from the Tn*21* transposon	This paper

phages λ.K were grown in *E. coli* TG-1 and *E. coli* K12 AB1157 cells respectively.

Vectors pTZ57R, pUC19, and pACYC184 were used. Genes of the *merRTPFADL* operon (about 4000 bp Tn*5053* DNA fragment) were introduced into the pACYC184 vector by the ClaI–SalI sites in order to obtain the pAC-mer hybrid plasmid. The *ardD* gene of the transposon Tn*21* was introduced into the pTZ57R vector using the following primers:

tniTn21D 5'-GTATAAACCGTCCAAGAAATC-GT-3' and

tniTn21R 5'-GACCAAACTATCGTGCGGGCG-3'.

The obtained hybrid plasmid was named pardD-Tn*21*.

Media, Enzymes, and Reagents

The LB medium was used that contained 1% tryptophan, 0.5% yeast extract, and 0.5% NaCl. 1.8% and 0.7% LB agar were used for the top and bottom layers of Petri dishes. The bacteria were grown on the LB medium at 30°C or on LB medium supplemented with 100 μg/mL ampicillin, 40 μg/mL kanamycin and 25 μg/mL chloramphenicol at constant stirring until the exponential growth phase.

Endonuclease cleavage, DNA fragment ligation, electrophoresis in agarose gel, and the isolation of DNA fragments were carried out as described in [13]. To perform cleavage and ligation, enzymes obtained from Fermentas (Lithuania) were used. *E. coli* cells were transformed by electroporation.

Table 2. Antirestriction activities of Tn*21*, Tn*5053*, Tn*5045*, Tn*501*, and Tn*402* transposons in *E. coli* AB1157 strain

Plasmid	Transposon	Gene	Restriction coefficient (*K*) of the λ.0 phage for the strain AB1157 r^+m^+*	Restriction alleviation factor, RA**
pUC19	–	–	1.0×10^{-4}	1
pKLH53.1	Tn*5053*	*merR* and *ardD*	4.0×10^{-2}	400
pUB837	Tn*21*	*merR* and *ardD'*	2.0×10^{-2}	200
pVS982	Tn*501*	*merR*	1.0×10^{-3}	10
pKLH402.1	Tn*402*	*ardD'*	1.5×10^{-3}	15
pKLH45.1	Tn*5045*	*chrR* and *ardD'*	2.0×10^{-2}	200

* Restriction coefficient (*K*) was estimated by the ratio of the λ.0 phage titer in the AB1157 strain to the titer of this phage in the TG-1 r^-m^- strain.

** Restriction alleviation factor $RA = K^+/K^-$, where K^+ is *K* for the AB1157 strain carrying the hybrid plasmid; K^- is *K* for the AB1157 strain carrying the pUC19 vector.

Here and in the Tables 3–5 the data shown are the results ofseveral repeats (at least 10). Mean and rounded data are shown.

Irradiation of the Phage and Bacteria

The suspension of the phage λ and bacteria in Tris buffer was irradiated with the lamp BUV-30 (254 nm). The UV dose was measured by the dosimeter UFD-4 with magnesium photocell. The adsorption of phages on nonirradiated and irradiated *E. coli* AB1157 cells was performed in TM buffer (0.05 M Tris-HCl, 0.01 M NaCl, and 0.01 M $MgSO_4$) at 37°C for 20 min. Phage–bacterium complexes were plated by the bilayer method adding the AB2463 indicator culture.

Estimation of Antirestriction and Antimodification Activities of the Transposons Tn*21*, Tn*5053*, Tn*5045*, Tn*501* and Tn*402*, and the ArdD and MerR Proteins

The antirestriction activities of the transposons and the MerR and ArdD proteins were measured using *E. coli* K12 AB1157 strain with EcoKI active type-I restriction-modification system. Antimodification activity of the transposon Tn*5053* was measured on *E. coli* K12 JM109 r^-m^+ strain with active EcoKI methylase. The procedure of antirestriction and antimodification activity measurement in proteins of the Ard family proteins is described in [14, 15].

RESULTS

Antirestriction Activities of the Transposons Tn*21*, Tn*5053*, Tn*5045*, Tn*501* and Tn*402*

Several different transposons, which contained *merR* and *ardD* genes in different combinations, were used to estimate antirestriction activity. Transposons Tn*21* and Tn*5053* contained both genes, whereas Tn*501* and Tn*402* contained only *merR* or *ardD* genes, respectively. Unlike the other transposons (besides the *ardD* gene), the Tn*5045* transposon also contained the operon that determined resistance to chrome (*chr* operon). It is noteworthy that transposons Tn*21*, Tn*5045*, and Tn*402* encoded the ArdD protein, which lacked 26 C-terminal residues compared to the ArdD encoded by Tn*5053* (in Table 2, the gene that encodes the truncated protein is designated as *ardD'*; amino acid sequences of the proteins ArdD and ArdD' are shown in Fig. 1). To assess antirestriction activities of the transposons Tn*21*, Tn*5053*, Tn*5045*, Tn*501,* and Tn*402*, hybrid plasmids were introduced into *E. coli* AB1157 cells. The results of these experiments are shown in Table 2.

It was shown that after introduction into *E. coli* cells the restrictionactivity of the transposons, which contained both *merR* and *ardD* genes, wasdecreased by more than 100 times (RA = 200–400), whereas in

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Tn5053 MGQGAVVDLQIVLTFRFQYPDAADVARIAPDQDGGQALAPGNPRLEGVGVVVDVVDD
Tn21   -R--T-GNF-VM-SL---D-T-----------K-AP-FT-R-----RI---------

Tn5053 KAGAGQGAADHRQEIDQKRGGAINDGRDANALHGLGPARGRRLEQAVVHGAVIGAYR
Tn21   ------R-PED--K---E-SS-CD---Y-DTR-----------------RT-V--D-
                                      147
Tn5053 QREPALQLAQRQGGHLLGIVLAALAGIGEGRPR***
Tn21   GC----***
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Fig. 1. Amino acid sequencies of ArdD protein of Tn*5053* and Tn*21* transposons.

Table 3. Additivity of antirestriction activities of *mer* operon (*merR* gene) and *ardD* gene of Tn*5053* and Tn*21* transposons in AB1157 strain

Plasmid	Gene	Restriction coefficient (*K*) of the λ.0 phage for AB1157 r^+m^+** strain	Restriction alleviation factor, RA**
pUC19	–	1.0×10^{-4}	1
pKLH53.1	*merR* and *ardD*	4.0×10^{-2}	400
pKLH53.100	*merR*	1.0×10^{-3}	10
pAC-mer	*merR*	1.0×10^{-3}	10
pTLORF5	*ardD*	2.0×10^{-3}	20
pAC-mer, pTLORF5	*merR* and *ardD*	2.0×10^{-2}	200
pUB837	*merR* and *ardD*'	2.0×10^{-2}	200
pardD-Tn21	*ardD*'	2.0×10^{-3}	20
pAC-mer, pardD-Tn21	*merR* and *ardD*'	1.8×10^{-2}	180

* The restriction coefficient (*K*) was estimated by the ratio of the λ.0 phage titer in the AB1157 strain to the titer of this phage in the TG-1 r^-m^- strain.

** The restriction alleviation factor $RA = K^+/K^-$, where K^+ is *K* for the AB1157 strain carrying the hybrid plasmid; K^- is *K* for the AB1157 strain carrying the pUC19 vector.

cells thatcontained transposons carrying only one of these genes the restrictionactivity was decreased by ten times (RA = 10–15). Interestingly, transposon Tn*5045*, which did not contain the *merR* gene, also demonstrated the RA value as high as mercury transposons Tn*21* and Tn*5053*, which contained this gene. It may be hypothesized that, in the genome of Tn*5045*, the role of *merR* is played by another gene. The most likely candidate for this role is the regulatory gene of the operon, which determines the resistance to chrome.

Additive Effect of Antirestriction Activities of the *mer* operon and the *ardD* gene

Plasmids pKLH53.100, pAC-mer, pTLORF5, and pardD-Tn21 were introduced into *E. coli* AB1157 both separately and in combination in order to assess the additivity of antirestriction activities of the *merR* and *ardD* genes (Table 3). It was shown that the separate introduction of the pKLH53.100, pAC-mer, pTLORF5, and pardD-Tn21 plasmids led to a decrease in restriction activity by 10–20 times. The combined introduction of the plasmids, which contained the *ardD* or *ardD*' gene, and the pAC-mer plasmid into the AB1157 strain resulted in a 180–200-fold decrease in restriction activity, which indicates the additivity of the effects of the *merR* and *ardD* genes.

The Effect of Mutations in *clpX* and *clpP* on Antirestriction Activity of the Transposons Tn*21*, Tn*5053*, Tn*5045*, Tn*501*, and Tn*402*

Protease ClpXP was shown to cleave the R subunit of the type I restriction-modification enzyme ($R_2M_2S_1$) when the enzyme is in contact with unmodified DNA [16–18]. This leads to the inhibition of restriction, because of the decrease in the level of endonuclease R subunit, though modification (methylase) activity (M_2S) remained unchanged. It may be suggested that antirestriction activity of the transposon Tn*5053* and, thus, proteins MerR and ArdD proteins, is connected with the activity of the ClpXP protease because the restriction activity of EcoKI decreased in the presence of Tn*5053*, while the modification activity remained unchanged [8]. To verify this hypothesis, we estimated the antirestriction activity of the Tn*21*, Tn*5053*, Tn*5045*, Tn*501*, and Tn*402* transposons and the plasmids carrying the *ardD*' and *merR* genes cloned in *E. coli* strains mutant by the *clpX* and *clpP* genes (Table 4). It was shown that the *clpX* mutation led to the almost complete absence of antirestriction activities of the transposons. The same result was obtained after the transposons were introduced into *E. coli* NK113 Δ*clpP* (data not shown).

Effect of *recA* and *recBC* Mutations on Antirestriction Activity of the Tn*5053*, Tn*5045*, and Tn*21* Transposons

The RA phenomenon, which was estimated based on the occurrence of unmodified DNA in the cell, was induced either as a result of the effect of DNA-tropic agents, which blocked replication (e.g., UV-RA [19–21]), or because of the effects of agents, which increased transitions and transversion frequency (for example, the effect of 2-aminopurine [22] or mutation in the *dnaQ* gene, which encodes the editorial ε subunit of the DNA polymerase III [17]) during DNA replication. Under UV irradiation, fully unmodified DNA (i.e., DNA in which both chains are not modified) was formed during homologous recombination upon the combination of two unchanged chains in the double stranded DNA fragment [23]. In this situation, the RecA-dependent SOS response was induced in the bacterial cell. The effect of restriction alleviation during UV irradiation was shown to depend on both the RecA and RecBC activity [19–21]. The fundamental

Table 4. Antirestriction activity of Tn*21*, Tn*5053*, Tn*5045,* Tn*501*, and Tn*402* transposons in *E. coli* NK114 Δ*clp*X strain that is defective by ClpXP protease

Plasmid	Transposon	Gene	Restriction coefficient (*K*) of the λ.0 phage for the strain NK114 r^+m^+*	Restriction alleviation factor, RA**
pUC19	No	–	2.0×10^{-5}	1
pKLH53.1	Tn*5053*	*merR* and *ardD*	2.0×10^{-5}	1
pUB837	Tn*21*	*merR* and *ardD*'	2.0×10^{-5}	1
pVS982	Tn*501*	*merR*	2.0×10^{-5}	1
pKLH45.1	Tn*5045*	*chrR* and *ardD*'	2.0×10^{-5}	1
pKLH402.1	Tn*402*	*ardD*'	2.0×10^{-5}	1
pardD-Tn21	No	*ardD*'	2.0×10^{-5}	1
pAC-mer	No	*merR*	2.0×10^{-5}	1

* Restriction coefficient (*K*) was estimated by the ratio of the λ.0 phage titer in the strain NK114 to the titer of this phage in the TG-1 r^-m^- strain.

** Restriction alleviation factor RA = K^+/K^-, where K^+ is *K* for the NK114 strain carrying the hybrid plasmid; K^- is *K* for the NK114 strain carrying the pUC19 vector.

Table 5. Effect of mutations in *recA* and *recBC* on antirestriction activity of Tn*21*, Tn*5053*, and Tn*5045* transposons

Strain	Transposon	Protein	Restriction coefficient (*K*) of the λ.0 phage for the AB2463 or JC7623* strains	Restriction alleviation factor, RA**
AB2463 *recA13*	pUC19	No	3.0×10^{-4}	1***
"	Tn*5053*	MerR and ArdD	3.0×10^{-3}	10
"	Tn*21*	MerR and ArdD'	3.0×10^{-3}	10
"	Tn*5045*	ChrR and ArdD'	3.0×10^{-3}	10
JC7623 *recB21recC22sbcB15*	pUC19	No	4.0×10^{-4}	1***
"	Tn*5053*	MerR and ArdD	4.0×10^{-3}	10
"	Tn*21*	MerR and ArdD'	4.0×10^{-3}	10
"	Tn*5045*	ChrR and ArdD'	4.0×10^{-3}	10

* Restriction coefficient (*K*) was estimated by the ratio of the λ.0 phage titer in the AB2463 or JC7623 strains to the titer of this phage in the TG-1 r^-m^- strain.

** The restriction alleviation factor RA = K^+/K^-, where K^+ is *K* for the strains AB2463 or JC7623 carrying the hybrid plasmid; K^- is *K* for the AB2463 or JC7623 strain carrying the pUC19 vector.

*** The restriction alleviation factor RA for the strains carrying the pUC19 was considered to be 1.

difference of the DnaQ-dependent and 2-aminopurine induced RA from UV-RA is its independence on RecA [24]. Therefore, we assessed the RA induced by the Tn*21*, Tn*5053*, and Tn*5045* transposons in the AB1157 strain as well as in *E. coli* AB2463 *recA13*; and *E. coli* JC7623 *recB21 recC22 sbcB15* (Table 5). It was shown that the RA effect induced by the Tn*21*, Tn*5053*, and Tn*5045* transposons was considerably decreased in *E. coli* AB2463 *recA13* and in the JC7623 *recB21recC22sbcB15* strains (RA = 10, which is 20–40 times lower than in the AB1157 strain). Therefore, the RA effect induced by transposons is determined to a significant degree by the formation of unmodified DNA. This process involves RecA and RecBC proteins that perform homologous recombination.

The transposons simultaneously with the RA transduction induce a slight SOS response in *E. coli* AB1157 $recA^+recBC^+$, which occurred as increased resistance to UV radiation (254 nm) of the phage λ upon the infection of the strain that contained the

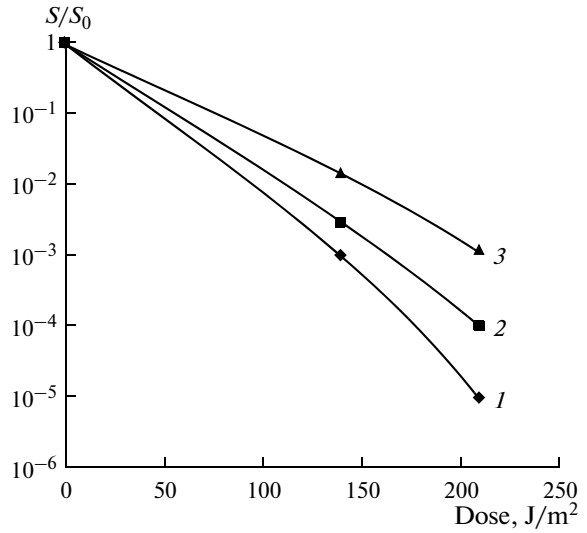

Fig. 2. Dependence of survival (S/S_0) of bacteriophage λ on the UV dose (254 nm). (*1*) Nonirradiated bacteria AB1157; (*2*) nonirradiated AB1157 bacteria (pKLH53.1); (*3*) UV-irradiated AB1157 bacteria (dose 20 J/m^2).

transposon (this effect is similar to the Weigle reactivation of the phage λ observed upon plating an irradiated phage on a UV-irradiated bacteria) (Fig. 2).

Effect of *dam* and *dnaQ* (*mutD*) on the Antirestriction Activity of the Tn*5053* Transposon

The loss of antirestriction activity by the transposons in the absence of the ClpXP protease means that the transposons induced some process that led to the formation of a considerable amount of unmodified chromosomal DNA in bacterial cells. It was previously shown that EcoKI restriction was significantly (approximately 20–100 times) downregulated in *E. coli* K12 strains mutant by the *dnaQ* (*mutD*) and *dam* genes [17, 25]. N.E. Murray et al. showed that these effects entirely depend on the presence of the ClpXP protease, which degrades the *R* subunit of EcoKI during the formation of the complex with unmodified DNA [16–18]. It might be suggested that proteins ArdD and MerR inhibit one of the following enzymes: Dam methylase, which is encoded by the *dam* gene, or the editorial ε subunit of DNA polymerase III, which is encoded by the *dnaQ* (*mutD*) gene. In this case, the presence of the Tn*5053* transposon in the mutant cell would not have increased the effect of restriction alleviation induced by the mutation. The results of this experiment are shown in Table 6.

It has been shown that, in the presence of the plasmid that carries the Tn*5053* transposon, the RA value in the mutant *E. coli dam::kan* strain, in which its own RA effect was 20, increased to 400 and became similar to that in the wild-type *E. coli dam*$^+$ strain. In the mutant *E. coli mutD::kan* strain, in which its own RA effect is 10, in the presence of the transposon Tn*5053*, the RA value increased by only five times and reached approximately 50. Therefore, the antirestriction activity of the Tn*5053* is not connected with the inactivation of Dam methylase, though partially determined by the editorial ε subunit of DNA polymerase III.

DISCUSSION

The dependence of antirestriction activity of the group of non-conjugative transposons Tn*21*, Tn*5053,* Tn*5045,* Tn*501,* and Tn*402* on ClpXP protease suggests that transposon carrying cells contain some amount of unmodified DNA (Table 4). Previously, it has been shown that the ClpXP protease specifically degrades the R subunit of the type-I restriction-modification enzyme ($R_2M_2S_1$) during the formation of a complex with fully unmodified DNA, thus decreasing the restriction activity of the enzyme without any effect on its modification (methylase) activity (M_2S_1) [16–18]. Unmodified DNA may be formed in a bacterial cell in result of SOS repair (for example, in UV-irradiated cells). In this case, the RA effect

Table 6. Antirestriction activity of the transposon Tn*5053* in the mutant strains of *E. coli dam::kan* and *E. coli mutD::kan*

Strain	Plasmid	Protein	Restriction coefficient (*K*) of the λ.0 phage for the AB1157, AB1157 *dam*, AB1157 *mut** strains	Restriction alleviation factor, RA**
AB1157	pUC19	–	1.0×10^{-4}	1
AB1157	pKLH53.1	MerR and ArdD	4.0×10^{-2}	400
AB1157 *dam*	pUC19	–	2.0×10^{-3}	20
AB1157 *dam*	pKLH53.1	MerR and ArdD	2.0×10^{-2}	400
AB1157 *mutD*	pUC19	–	1.0×10^{-3}	10
AB1157 *mutD*	pKLH53.1	MerR and ArdD	5.0×10^{-3}	50

* The restriction coefficient (*K*) was estimated by the ratio of the λ.0 phage titer in the AB1157 strain (either AB1157 *dam::kan* or AB1157 *mutD::kan*) to the titer of this phage in the TG-1 r^-m^- strain.

** The restriction alleviation factor RA = K^+/K^-, where K^+ is *K* for the strains carrying the hybrid plasmid; K^- is *K* for the strains carrying the pUC19 vector.

depends entirely on the activities of both the RecA and RecBC proteins [19–21]. Unmodified DNA may also be formed during replication if a number of incorrect nucleotides is accumulated, e.g., as a result of transitions and transversions in the *dnaQ* mutant, in which the editorial ε subunit of DNA polymerase III is not active, or during the replication in the presence of 2-aminopurine. In this case the RA effect does not depend on the recombination proteins [17, 24]. The RA effect, which is induced in a bacterial cell by Tn*21*, Tn*5053,* Tn*5045,* Tn*501,* and Tn*402* non-conjugative transposons is complex in nature. Based on the experimental data on the partial dependence of the transposon induced RA effect on both the RecA and RecBC proteins and the editorial ε subunit of DNA polymerase III, it may be suggested that unmodified DNA is formed as a result of both recombinational repair and the accumulation of transitions and transversions during DNA replication.

What is the role of RA induced by the products of the *merR* and *ardD* genes in the "life cycle" of non-conjugative transposons? It may be suggested that, when inducing the formation of a pool of unmodified chromosomal DNA in a bacterial cell, MerR and ArdD proteins not only initiate the RA effect, but also support transposition. It was shown that UV irradiation, as well as other types of cell treatment, which induce DNA damage, considerably increase (by dozens of times) the frequency of intracellular transpositions of different groups of transposons [26–29]. The presence of *merR* and *ardD* genes, the products of which disturb modifications of the host DNA, may facilitate normal transposition in cells, which did not undergo treatment with DNA-tropic agents. The presence of open reading frames homologous to *ardD* in a large group of non-conjugative complex transposons and integrons demonstrates the important role of the ArdD proteins in their "life cycle." It is noteworthy that, in most transposons and integrons, including Tn*21*, open reading frame encode the ArdD' protein, which is shorter than the ArdD of the transposon Tn*5053* by 26 amino acid residues. However, this does not decrease its antirestriction activity. It should be noted that the amino acid sequence of the ArdD' protein contains far less glycine than the ArdD of Tn*5053* (12 instead of 24).

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