Conflicts Targeting Epigenetic Systems and Their Resolution by Cell Death: Novel Concepts for Methyl-Specific and Other Restriction Systems

KEN Ishikawa¹, ERI Fukuda¹, and ICHIZO Kobayashi¹,²,³,*

Department of Medical Genome Sciences, Graduate School of Frontier Sciences, University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo, Japan¹; Graduate program in Biophysics and Biochemistry, Graduate School of Science, University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo, Japan² and Institute of Medical Science, University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo, Japan³

*To whom correspondence should be addressed. Tel. +81 3-5449-5326. Fax. +81 3-5449-5422. E-mail: ikobaya@ims.u-tokyo.ac.jp

Edited by Katsumi Isono
(Received 26 August 2010; accepted 30 September 2010; published online 8 November 2010)

Abstract

Epigenetic modification of genomic DNA by methylation is important for defining the epigenome and the transcriptome in eukaryotes as well as in prokaryotes. In prokaryotes, the DNA methyltransferase genes often vary, are mobile, and are paired with the gene for a restriction enzyme. Decrease in a certain epigenetic methylation may lead to chromosome cleavage by the partner restriction enzyme, leading to eventual cell death. Thus, the pairing of a DNA methyltransferase and a restriction enzyme forces an epigenetic state to be maintained within the genome. Although restriction enzymes were originally discovered for their ability to attack invading DNAs, it may be understood because such DNAs show deviation from this epigenetic status. DNAs with epigenetic methylation, by a methyltransferase linked or unlinked with a restriction enzyme, can also be the target of DNases, such as McrBC of Escherichia coli, which was discovered because of its methyl-specific restriction. McrBC responds to specific genome methylation systems by killing the host bacterial cell through chromosome cleavage. Evolutionary and genomic analysis of McrBC homologues revealed their mobility and wide distribution in prokaryotes similar to restriction–modification systems. These findings support the hypothesis that this family of methyl-specific DNases evolved as mobile elements competing with specific genome methylation systems through host killing. These restriction systems clearly demonstrate the presence of conflicts between epigenetic systems.

Key words: intragenomic conflict; programmed cell death; epigenetic DNA methylation; restriction–modification system; McrBC

1. Introduction

Recent studies have revealed that epigenetic systems are involved in many aspects of biological processes. Epigenetics is often involved in conflict between genetic units. For example, epigenetic DNA methylation is associated with the silencing of selfish mobile elements and with the imprinting of alleles inherited from a particular parent. This review article introduces a new concept in epigenetics: intragenomic conflict with epigenetic systems. Such conflicts become apparent when they are resolved by cell death. The death takes place after decrease (Sections 5–8) or increase (Sections 9–15)¹–³ in epigenetic DNA methylation (Table 1). Our emphasis will be on systems involving DNases, including those that are methyl specific.

2. Epigenetic DNA methylation and its significance

In this review, the term epigenetic is defined as ‘not genetic, but heritable through DNA replication’ and is
used to distinguish among three modes of DNA methylation: (i) genetic methylation, for example, in the biosynthesis of dTMP from dUMP, with subsequent incorporation into DNA by the replication machinery; (ii) epigenetic methylation, as in 5-methylcytosine (m5C), N4-methylcytosine (m4C), and N6-methyladenine (m6A), which are inherited by maintenance methylation after DNA replication; and (iii) non-genetic and non-epigenetic methylation, e.g., O6-methylguanine. The non-epigenetic and the non-genetic DNA methylation in O6-methylguanine are known to trigger cell death.4

Another class of DNA modification involves the use of a base other than A, T, G, and C in DNA. For example, some bacteriophage genomes carry hydroxymethylcytosine instead of cytosine,5 and dUMP is often incorporated in place of dTMP into some bacteriophage genomes.6

In eukaryotes, epigenetic DNA methylation plays roles in chromatin organization, gene expression, and genome maintenance, and its disturbance is related to human diseases.7–9 In prokaryotes, it is crucial for processes including cell-cycle regulation, transcriptional regulation, and host-pathogen interaction.10–13 It is also involved in silencing selfish genetic elements and other aspects of intragenomic conflicts in eukaryotes14 and in prokaryotes (this review, see below).

Switching on and off of DNA methyltransferase through phase variation in bacteria can change the entire transcriptome.15 Experimental alteration of epigenetic DNA methylation systems in prokaryotes can cause a variety of changes.10,15–18 Horizontal gene transfer between prokaryotic genomes is common,19 and the DNA methyltransferase genes, in particular, are known to frequently undergo this type of transfer.20–24 The DNA methyltransferases could, therefore, represent potential threats to the epigenomic integrity of prokaryotic genomes.

3. Restriction–modification systems

In prokaryotes, many epigenetic DNA methyltransferases are paired with a restriction enzyme.25 Restriction enzymes are DNA endonucleases that recognize specific DNA sequences and introduce a

| Change in DNA methylation | Condition | Condition in detail | Gene involved | Note | References |
|---------------------------|-----------|---------------------|---------------|------|------------|
| Decrease                  | Loss of DNA methyltransferase | Loss of restriction—modification gene complex | Type II restriction—modification genes | Post-segregational killing; *Escherichia coli* | 52,53,62 |
|                           | Inactivation of DNA methyltransferase | Type II modification gene (for *ecoRIIM*) | *Escherichia coli* | 65 |
|                           | Loss of DNA methyltransferase gene | *dam* | DNA replication initiation defect in *Vibrio cholerae* | 12 |
|                           |                      | *ccrM* | DNA replication and cell-cycle defect in *Caulobacter crescentus* | 12 |
|                           |                      | *dnmt1* (DNA methyltransferase I) | Knockout, in mice embryos | 16 |
| Through DNA metabolism    | Base substitution mutation | Type I restriction—modification genes (*ecoKI, ecoR124I, in the absence of restriction alleviation*); Type II restriction—modification genes (*ecoRI, ecoRIM*) | 75,78,79 |
|                           | Interstrand crosslink repair | Type II restriction—modification genes (*ecoRI, ecoRIM*) | 81 |
| Increase                  | Exogenous expression of DNA methyltransferase | *mcrBC* | Indirect evidence | 127 |
|                           |                      | *mrr* | Mouse gene in fly | 2 |
|                           |                      | *dnmt1* and *dnmt3a* | Mouse gene in fly; mouse gene in frog | 2,3 |
|                           |                      | *dnmt3a* | Mouse gene in frog | 3 |

Table 1. Programmed cell death and changes in epigenetic DNA methylation
double-strand break (Fig. 1A). This activity restricts establishment of invading DNAs that lack proper DNA methylation, such as bacteriophage DNA genomes, plasmids, and DNA fragments delivered through natural transformation machinery (Fig. 1B). The potentially lethal cleavage of cellular DNA in cells that harbour a restriction enzyme is prevented by epigenetic DNA methylation by the cognate DNA methyltransferase that recognizes the same sequence as the restriction enzyme (Fig. 1A and B). Genes encoding the restriction enzyme and the methyltransferase are often located next to each other and form a unit called a restriction-modification system. Restriction-modification systems are classified into four types, Type I, II, III, and IV, based on their genetic and biochemical characteristics.

3.1. Type II systems
Type II restriction enzymes bind to a recognition sequence and cleave DNA in their vicinity and are frequently used in DNA engineering. For example, EcoRI, BamHI, and PvuII are Type II enzymes. Many variants are classified into subtypes within this type, based on biochemical characteristics. In many subtypes, restriction activity is present in one enzyme molecule, whereas modification activity is present on the other. Restriction enzymes in this class are divergent in amino acid sequence and three-dimensional structure and can be also classified based on these features.

3.2. Type I systems
Type I and III enzymes are composed of multiple subunits, and their restriction and modification activities depend on their subunit composition. Type I restriction enzymes are composed of three subunits, S, M, and R. The S subunit recognizes a specific DNA sequence. A complex of M and S subunits exhibits methyltransferase activity at the recognition site. The joining of the R subunit to this complex is essential for endonuclease activity. After binding to an unmodified recognition sequence, the restriction enzyme complex translocates DNA towards itself from both directions in a reaction coupled to ATP hydrolysis (Fig. 2A). When two restriction enzyme complexes collide, DNA cutting is triggered. This is consistent with an in vivo observation. Cleavage can occur also through interaction of the translocating restriction enzyme complex with a Holliday junction, a single-strand gap, a single-strand nick, or a long branch (Fig. 2B; see Section 8).

Type I restriction-modification enzymes have two modes of action that are controlled by the
The methylation state of their recognition sequence. If the sequence is fully methylated, the enzyme complex does not bind. When the sequence is hemimethylated, the methyltransferase complex catalyzes an efficient methyltransfer reaction to the other strand. When the sequence is unmethylated, the restriction enzyme complex is formed and translocation begins, leading to cleavage.

3.3. Type III systems

Type III restriction enzymes are composed of two subunits: Mod (for modification) and Res (for restriction). The Mod subunit has DNA methyltransferase activity, and the Mod–Res complex has restriction activity. When the restriction enzyme complex binds to an unmethylated site, it cleaves DNA through interaction with another restriction enzyme complex on the same DNA. This process is dependent on ATP hydrolysis. The cleavage mechanism is not yet clear, although diverse and sometimes mutually contradictory models have been proposed.

3.4. Type IV systems

Type IV systems contain a class of enzymes that cleave DNA only when the recognition site is methylated. In *Escherichia coli*, McrA, McrBC, and Mrr are enzymes in this class that show different restriction spectra. McrBC, the best characterized of this class, is described in detail below (Section 9). Although McrA and Mrr are believed to be endonucleases, their DNA cleavage activities have not been observed in vitro.

4. Mobility of restriction–modification systems

The DNA methyltransferase genes frequently undergo horizontal transfer. The transfer of an epigenetic DNA methyltransferase gene can be the initial step of the genetic conflicts described in following sections (Sections 11–15).

Many DNA methyltransferase genes of restriction–modification systems show signs of mobility by various criteria. Horizontal transfer between distantly related prokaryotes has been demonstrated by molecular evolutionary analyses. Restriction–modification systems are often found on mobile elements such as plasmids, bacteriophages, integrative conjugative elements, transposons, genomic islands, and integrons. Some restriction–modification systems appear to behave as a mobile unit without being linked to another mobile unit. Examination of the genomic neighbourhood of restriction–modification gene homologues and comparison with closely related genomes also provide evidence for their mobility and association with genome rearrangements. Restriction–modification systems can insert into an operon-like gene cluster, or they can insert into a genome with a long (~100 bp) target duplication. They can substitute for a genomic region, or transpose into a different genomic locus. They are sometimes linked to large chromosomal inversions.

Recently, our group conducted systematic genome comparisons and genome context analysis on fully sequenced prokaryotic genomes to detect restriction–modification-linked genome rearrangements. Restriction–modification genes were frequently found to be linked to mobility-related genes such as integrase and transposase homologues. Restriction–modification genes were found to be flanked by direct and inverted repeats at a significantly higher frequency than control genes. ‘Insertion accompanied by long target duplication’ was observed for I, II, III, and IV restriction types. Several restriction–modification genes were found to be flanked by long, imperfect inverted repeats, just as transposable genes are in classical DNA transposons. Some of these had apparently inserted into a genome with a short target duplication, similar to DNA transposons.
functional. A mobility-based search for novel restriction enzymes detected a previously uncharacterized family of DNA-interacting proteins. The mobility of McrBC is described in Section 13.

As described in Section 5, Type II restriction–modification systems contribute to their own genetic stability and to the stability of genes linked to them. Their linkage to a mobile element would be mutually beneficial. Restriction–modification systems would stabilize the element, and the mobile element would contribute to spreading of the restriction–modification systems.

The behaviour of Type II restriction–modification systems as mobile elements is further suggested by their amplification and involvement in genome rearrangements under laboratory conditions, as well as by their mutual competition and regulation of gene expression. Some restriction–modification systems have evolved regulatory systems to suppress their potential for host killing. When the system enters a new host, host cell killing is prevented by initial expression of the methyltransferase, with delayed expression of the restriction enzyme. This regulation can cause a conflict between two epigenetic systems that is similar to phage exclusion, which is described in Section 6.

5. Host attack by Type II systems upon gene loss

As described above, epigenetic DNA methyltransferase genes often form a restriction–modification system in prokaryotes, and epigenetic conflicts are coupled to the behaviour of the restriction–modification systems.

Some Type II restriction–modification systems cause chromosomal cleavage of their host cells when their genes are eliminated, for example, by a competitor genetic element (Fig. 1C, without competitor; Fig. 3A, with a competitor). When a restriction–modification system is stably maintained in the cell, the restriction enzyme does not cleave the genomic DNA because of protection through epigenetic methylation by the cognate methyltransferases. However, when the restriction–modification gene complex is lost from the cell, the concentration of the restriction and modification enzymes is decreased through cell division, resulting in unmethylated chromosomes. The remaining restriction enzyme molecules cleave the unmethylated recognition sequence and cause cell death. The net result is survival of cells that were not invaded by the competitor (Fig. 3A). This process is called ‘post-segregational killing’ or ‘genetic addiction’. Similar host attack can take place after inactivation of the modification enzyme. Host killing forces cells to maintain their genes (or enzyme activity) and the epigenetic status conferred by the methyltransferase (Figs 1C and 3A).

This cell death process may be a composite of host defence and suicide responses. Recent studies revealed a common pathway of stress-induced cell death in bacteria. Transcriptome analysis during post-segregational death programmed by a Type II restriction–modification system revealed its similarity to death caused by several antibiotics. Gene products that program bacterial cell death, such as the restriction enzymes discussed here, are likely to work in the upstream of the common cell death pathway. In other words, action of the death genes may depend on the common cell death pathway of the host.

Although the pathway may facilitate host cell death, the repair and tolerance mechanisms work to facilitate survival. In general, chromosomal cleavage by cellular DNases is prevented in several ways: by inhibitor binding, compartmentalization, proteolysis, DNA modification, or DNA structure specificity. Indeed, host killing by restriction–modification systems after gene loss is not always detectable because hosts have apparently adapted various ways to counteract it. Recombination repair of chromosomal breakage can reduce the lethal effects of chromosome cleavage. Host killing by EcoRII restriction–modification system is suppressed by a solitary methyltransferase,
Restriction in Epigenetic Conflicts

Dcm, which recognizes the same sequence.⁷⁰ These host defence systems against restriction–modification systems cannot, however, prevent host genome methylation and its potentially deleterious effects, which are the focus of the latter half of this review (Sections 11–15).

Many Type II restriction enzymes show star activity or promiscuous activity on sequences other than their recognition sequence protected by cognate methylation.⁷¹ It is not known whether these lead to genome cleavage or cell death under some condition.

6. Conflicts between two epigenetic systems (Type II restriction–modification systems)

Restriction–modification systems are mobile genetic elements, as discussed above (Section 4), and Type II restriction–modification systems are in potential conflict with competitor restriction–modification systems encountering through their mobility. Type II systems can be also considered epigenetic systems because their action is mediated by epigenetic DNA methylation. In this section, we describe conflicts involving Type II restriction–modification systems, which can be considered conflicts between epigenetic systems.

One restriction–modification system can block the post-segregational killing potential of another restriction–modification system with the same recognition sequence (Fig. 1C). When two Type II restriction–modification systems carrying a methyltransferase with the same sequence specificity are present in the same cell, interference between the two systems affects post-segregational killing. Loss of one restriction–modification gene complex does not lead to cell killing, because the methyltransferase of the other restriction–modification protects the genomic recognition sequences from attack by the restriction enzyme of the first restriction–modification. This prediction was verified experimentally.⁵⁴ This within-host competition for recognition sequences may have driven the evolution of the individual specificity and the collective diversity in target sequence recognition by restriction–modification systems. Such incompatibility, or competition for specific sequences along the genome, would result in specialization of each of these selfish units to each of these diverse sequences. This may represent an example of ‘competitive exclusion’ in biological evolution, which drives adaptation of each of many species to one of many small ecological niches in an exclusive way. We imagine that the ecological niche of an RM system is the recognition sequence.⁵⁴

We also analyzed intrahost competition between two RM gene complexes when the recognition sequence of one was included in that of the other. When the EcoRII gene complex, recognizing 5'-CCWGG (W = A and T), is lost from the host, the SsoII gene complex, which recognizes 5'-CCNGG (N = A, T, G, and C), will prevent host death by protecting 5'-CCWGG sites on the chromosome. However, when the SsoII (CCNGG) gene complex is lost, the EcoRII (CCWGG) gene complex will be unable to prevent host death through attack by SsoII on 5'-CCSGG (S = C and G) sites. These predictions were verified in our experiments.⁷²

Through this type of conflict, a toxic restriction–modification system may be replaced by a less toxic restriction–modification system with the same sequence specificity, or even by a solitary methyltransferase lacking a restriction enzyme gene. This explains why the chromosomes of E. coli and related bacteria encode Dcm, a solitary methyltransferase that protects the genome from attack by the EcoRII restriction–modification system found on plasmids. This effect is called molecular vaccination.⁷⁰ In these cases, a conflict between two epigenetic systems inhibits host cell death. In the following case, a conflict between two epigenetic systems (e.g. restriction–modification systems) leads to cell death, similar to phage exclusion or post-segregational killing.

When a Type II restriction–modification system establishes itself in a new host, it first expresses the modification gene to protect recognition sequences in the genome and prevent cell killing, before expressing the restriction gene. Here, the accumulation of a regulatory protein—the modification enzyme itself or a C regulatory protein—leads to the expression of the restriction enzyme. When a resident restriction–modification system has the same specificity in the establishment-regulating mechanism, the regulatory protein of the resident restriction–modification system that induces restriction enzyme expression may act on the invading restriction–modification system. This forces the invading restriction–modification system into the premature expression of the restriction enzyme gene in the absence of prior expression of the modification enzyme gene. This kills the host, aborting the establishment of the incoming restriction–modification system. The overall effect is similar to phage exclusion (Fig. 6) or post-segregational killing with a competitor genetic element (Fig. 3). This predicted model has been experimentally verified⁵⁵ and termed super-infection exclusion or apoptotic mutual exclusion. This mutual competition between restriction–modification systems may have driven the evolution of specificity in the mechanisms for regulation of establishment.
7. Genomic restriction attack following loss of DNA methylation through DNA damage repair

In addition to the loss of epigenetic systems (Sections 5 and 6), epigenetic DNA methylation may be decreased by DNA damaging agents through the DNA metabolism processes of replication, recombination, and repair. This is supported by many studies on restriction alleviation of Type I RMs.

Restriction alleviation is the phenotypic decrease in restriction activity on invading DNA that can be induced by DNA damaging agents or occurs constitutively in some bacterial mutants. The underlying mechanism varies by a restriction enzyme subtype. Evidence suggests that restriction alleviation is a mechanism for protecting chromosomes from restriction at a newly generated replication fork that produces unmethylated restriction sites. Indeed, chromosome breakage leading to cell death is observed when the restriction alleviation effect is lost for EcoKI and EcoR124I (Table 1). Thus, restriction alleviation can be regarded as an indirect sign of a decrease in epigenetic methylation.

The molecular mechanisms that lead to loss of EcoKI methylation by ultraviolet light have been suggested based on its dependence on nucleotide excision repair function and on the primosome assembly activity of the PriA protein. DNA double-strand breakage may occur through replication progression on a single-stranded DNA gap generated by nucleotide excision repair. The double-stranded DNA end will be subject to RecBCD processing, followed by homologous pairing and D-loop formation by RecA, and establishment of a new replication fork by a primosome. Through this process, new DNA strands can pair and form unmethylated recognition sites.

Base-substitution mutation represents another route for the loss of epigenetic methylation. The base analogue 2-aminopurine (2-AP) can be incorporated into DNA strands, forming a mismatched base pair that causes a T→A transition after two rounds of replication. This mutagenesis generates additional unmethylated recognition sequences for some restriction—modification systems with a methyltransferase protecting DNA with an m6A methylation. In fact, when 2-AP is added to cultures, the EcoKI restriction enzyme cleaves the bacterial chromosome in vivo, causing cell death if the restriction alleviation mechanism is absent. Similarly, EcoR124I, from a Type I restriction—modification system, and EcoRI, from a Type II restriction—modification system, cause cell death, depending on their restriction activity, under these conditions (Table 1).

The lethal effect of mitomycin C, a DNA cross-linker, is enhanced by EcoRI restriction—modification. A possible explanation is that DNA cross-link repair removes DNA methylation at the restriction sites (Table 1). Other observations indirectly suggest that loss of epigenetic methylation can occur in certain mutants with replication fork crowding, specifically dam, topA, rnhA, and recG for EcoKI; rnhA and recG for EcoRI 241. Thus, DNA damage repair processes and genome instability can cause loss of epigenetic methylation, which is detected by the activity of restriction—modification systems.

8. Cleavage of DNA replication forks by Type I restriction enzymes

As discussed above, loss of epigenetic DNA methylation is often coupled to DNA replication, which suggests that chromosomal DNA cleavage by restriction enzymes may be related to DNA replication. Association of DNA replication and Type I restriction by EcoKI is observed in phage restriction. Direct interaction between the M subunit of EcoKI and DnaB, a central component of the DNA replication machinery, was reported in a large-scale E. coli protein—protein interaction analysis.

Recently, we demonstrated that the Type I restriction enzyme EcoRI241 cleaves model replication forks at their branch point in vitro. Cleavage was dependent on the presence of a recognition sequence on one of the arms and was inhibited by its hemimethylation. The enzyme cleaves the arm carrying the recognition sequence, but does not cleave the arm lacking the recognition sequence. The recognition sequence must be a long distance (300 bp) from the branch for efficient cleavage. These results are consistent with a reaction mechanism in which the enzyme binds to DNA at the recognition sequence and starts tracking along the DNA. It cleaves DNA when it encounters a branch point (Fig. 2B).

Cleavage at a replication fork to remove a branch provides an explanation for the association of DNA replication and restriction, and the recombination repair of restriction damage observed after a single infection by a phage genome. The restriction enzyme would recognize a site on a daughter chromosome and track along the DNA until it reaches a moving replication fork. At the fork, the enzyme cleaves one branch, leaving replicated and broken daughter chromosomes with a long overlap sufficient in some cases for repair by homologous recombination.

This fork cleavage may take place on chromosomal DNA under the conditions of extra replication initiation described above. From an exposed (unmethylated) recognition sequence, the restriction
enzyme would track on the DNA. If the fork is moving forward during replication, DNA breakage might not occur. However, if the enzyme meets an arrested replication fork, breakage would cleave off one arm (Fig. 2B), possibly leading to cell death or to another round of repair through recombination and replication. Elimination of a cell with unstable, damaged DNA would lead to maintenance of intact genomes, which is a recurrent theme of this review. The race between the replication fork and the restriction enzyme would help a cell to collect multiple types of information related to the life or death decision.

In brief, we hypothesize that a Type I restriction—modification may monitor the epigenetic DNA methylation level together with the DNA replication machinery. This restriction—modification system maintains a proper methylation level by eliminating cells with unusual levels through DNA replication fork cleavage and complements systems that repair damaged genomes. This hypothesis explains the unusual enzymatic activity of Type I restriction endonucleases, which translocate along the DNA before cleaving it. This process may balance death and revival, depending on at least two criteria: the level of epigenetic methylation and a replication condition that is either moving or stalled.

The other DNA-tracking restriction enzymes, Type III and Type IV, might have some interaction with a replication fork. A fraction of Type III restriction cleavage of incoming phage DNA likely takes place after passage of the replication fork in vivo.

In contrast to cell death in response to a decrease in genome DNA methylation, we discuss cell death in response to its increase in the latter half of this review.

9. McrBC, a methyl-specific DNA endonuclease (Type IV restriction enzyme)

McrBC, a methyl-specific DNA endonuclease (Type IV restriction enzyme) in E. coli, was first recognized for its restriction of some bacteriophages (see next section). McrBC is encoded by two genes, mcrB (rglB) and mcrC. The mcrB gene encodes two different protein forms, McrBL (a larger, full-length form) and McrBS (a smaller form). These proteins share the same amino acid sequence except for 161 amino acids at the N-terminus that are missing in McrBS. McrBL and McrC are sufficient for methyl-DNA cleavage activity, and McrBS is involved in activity modulation.

McrB is composed of two functional domains. The N-terminal domain specifically binds to a methylated recognition site. The C-terminal domain has GTPase activity and includes three GTP-binding motifs. McrC is the endonuclease subunit with a PD-(D/E)×K motif. This is the most abundant motif in Type II restriction enzymes characterized thus far and is often found in DNA repair and recombination-related enzymes.

The recognition sequence of E. coli McrBC is composed of two sites, in the form of RmC N40–2000 RmC (where R is A or G). DNA double-strand cleavage occurs between the two sites preferably at ~30 bp inward from one of the sites (Fig. 4A). Methylation does not need to be on the same DNA strand, so the two sites do not need to be in a particular orientation. Similar to Type I restriction enzymes, efficient cleavage by the enzyme requires two recognition sites, except in the case of a circular DNA substrate with a single recognition site. McrBC binds to the specific recognition site RmC through the N-terminal DNA-binding domain of McrBL. Double-stranded DNA cleavage occurs in vivo.
through interaction of two McrBC complexes on the
DNA.\textsuperscript{99} The interaction is facilitated through translo-
cation of the enzyme complexes along the DNA rather than through DNA looping.\textsuperscript{90} GTP and Mg\textsuperscript{2+} are required cofactors for the cleavage reaction.\textsuperscript{99}

Comparison of intragenomic paralogues revealed possible diversification in sequence recognition in
McrBC homologues.\textsuperscript{1} Some genomes, such as the
Deinococcus radiodurans R1 genome, contain two
mcrBC homologues: one may be on a plasmid whereas the other on the chromosome.\textsuperscript{1} Alignments of
intragenomic McrB homologue pairs revealed
amino acid sequence divergence in the N-terminal
region that is involved in DNA binding\textsuperscript{102} suggesting
evolutionary shifts in DNA sequence specificity.\textsuperscript{1} This
parallels the diversity in sequence recognition in
Type II restriction and modification enzymes.

10. Biological role of McrBC

The biological significance of McrBC was first recog-
nized in the restriction of invading bacteriophage
genomes carrying hydroxymethylcytosine instead of
cytosine (Fig. 4B).\textsuperscript{107,108} Bacteriophages carrying
this unusual base are rare.\textsuperscript{109,110} McrBC may also
protect cells against infection by methylated DNAs,
such as viral genomes and plasmids, by directly cleav-
ing invading DNA. However, such methylated DNAs
are not usually strongly restricted by McrBC (Fig. 4B).\textsuperscript{1,42,111} This suggests that defence against
invading methylated DNA through direct attack may
not be the primary role of McrBC.

Therefore, similar to Type II and I restriction–modi-
fication systems (see above), we hypothesized that
McrBC may mediate a suicidal response to epigenetic
DNA methylation and may maintain the epigenomic
status. The behaviour of McrBC upon DNA methyl-
transferase invasion might be similar to that of
Type II restriction–modification, as illustrated in
Fig. 3B. When such a methylation system enters the
cell (or becomes activated) and begins to methylate
the host genome, McrBC would sense these epige-
netic changes and trigger cell death through chromo-
somal cleavage. Intact (unmethylated) genomes with
mcrBC genes would survive in neighbouring clonal
cells,\textsuperscript{1} and the host killing could also contribute to
an increase in their gene frequency.

Recently, we demonstrated that McrBC-mediated
cell death through cleavage of methylated chromo-
somes occurs upon entry or induction of a methyl-
transferase gene and aborts its establishment or
activation.\textsuperscript{1} Our genome informatics analysis sup-
ported the hypothesis that, during evolution, McrBC
has behaved as a mobile element.\textsuperscript{1} Therefore, main-
tenance of McrBC enzymes cannot be explained only
by the restriction of bacteriophages containing hydro-
xymethylcytosine. These are discussed further in the
following sections.

11. McrBC-mediated host cell killing through
chromosomal cleavage upon genome
methylation

Several reports found that phages or plasmids car-
rying a DNA methyltransferase gene cannot be prop-
gagated in an mcrBC\textsuperscript{+} strain of E. coli (Fig. 4B).\textsuperscript{112} Whether the block to propagation is due to repeated
methylation of the introduced DNA and subsequent
cleavage\textsuperscript{112} or to host genome methylation and its
cleavage has not been addressed. Fukuda et al.\textsuperscript{1}
demonstrated that McrBC inhibits establishment of
the gene for the DNA methyltransferase PvuII
(M.PvuII, CAC\textsuperscript{m4}CTG) in E. coli. Establishment of a
plasmid carrying this gene but lacking its recog-
nition sequence was inhibited. This result suggests
that the presence of methylated sites on the
transferred DNA is not required for McrBC-depend-
ent inhibition,\textsuperscript{1} favouring the latter possibility
that host genome cleavage accompanied by cell
death inhibits the establishment of the methyltrans-
ferase gene.

The underlying mechanism of the cell death was
revealed by observing chromosomal DNA of E. coli
infected with lambda phage carrying the M.PvuII
gene.\textsuperscript{1} Accumulation of huge linear DNAs cor-
responding to broken chromosomes, and of smaller
DNAs of variable size was observed, which likely
reflected chromosome degradation. mcrBC-depen-
dence strongly suggested that M.PvuII-mediated
chromosomal methylation triggered chromosomal
cleavage by McrBC, followed by chromosomal degra-
dation. This, in turn, indicates that inhibition of the
phage multiplication (restriction) is caused by host
death.\textsuperscript{1} This kind of conflict between DNA methyl-
transferase genes carried by bacteriophages and
methyl-specific restriction enzymes are biologically
relevant because DNA methyltransferase genes are
often found in bacteriophage genomes.\textsuperscript{25,113–116}
The resolution by cell death may contribute to
increase in the frequency of the restriction gene as
described in Section 14.

Induction of the M.PvuII in cells also led to chromo-
somal methylation followed by McrBC-mediated clea-
vage and cell death. Furthermore, a close correlation
was seen between methylation, cleavage, and death.
By mutant analysis, the SOS response and RecA/
RecBCD-mediated DNA recombination and repair
were found to affect cell death or survival upon
McrBC activation on the methylated genome. These
observations are consistent with the hypothesis that
chromosomal methylation leads to McrBC-mediated lethal cleavage.

In addition to M.PvuII, M.SinI (GGWm5CC) and M.MspI (m5CCGG) causes McrBC-dependent cell death, whereas M.SsoII (Cm5CNGG) does not. These results are consistent with the Rm6C sequence specificity of McrBC observed in vitro. McrBC has the potential to act as a defence system against many DNA methyltransferases with an appropriate specificity. Such conflicts between McrBC and invading epigenetic DNA methylation systems may have driven diversification of sequence recognition by the methyltransferases and by the McrBC family (see above and below), just as competition between Type II restriction-modification systems may have driven diversification of their sequence recognition (Section 6).

12. Linkage of McrBC with DNA methyltransferase genes defines epigenomic status

Epigenetic DNA methylation has crucial roles in cell-cycle regulation, transcriptional regulation, transposition of mobile elements, and host-pathogen interaction, as discussed above. Methyl-DNA-specific DNAses could contribute to maintenance of specific epigenomic states by inhibiting establishment of invading epigenetic DNA methylation systems (or by inhibiting their expression) through cell death (Fig. 5). This is comparable to maintenance of an epigenome status by Type II restriction-modification systems through cell killing.

The mcrBC homologues are frequently linked to DNA methyltransferase genes, as first noted for E. coli. The methyltransferase is frequently from a Type I restriction-modification system, and less often from a Type IIG restriction-modification system. The linked methyltransferase is expected to have a specificity that does not create a target for the McrBC nuclease. This implies that the McrBC will eliminate methyltransferases with specificity different from its linked methyltransferase. The base specificity of Type I modification enzymes, i.e. m6A methylation as opposed to m4C and m5C of McrBC, is consistent with this hypothesis. The mcrBC genes and linked methyltransferase genes can be regarded as units that force an epigenome status in competition with other, invading epigenetic DNA methylation systems (Fig. 5). Although a linked epigenetic DNA methylation system provides an epigenomic methylation, establishment of another invading epigenetic DNA methylation system is inhibited by McrBC-mediated cell killing. McrBC plays the role of a judge forcing maintenance of an epigenomic order by eliminating cells attracted by another epigenomic order (Fig. 5).

Considering the crucial roles of epigenetic DNA methylation in biology, systems to maintain epigenome integrity by cell death are expected to be broadly conserved. Failure of the maintenance mechanism might be related to the generation and adaptation of cancer cells, in which epigenomic DNA methylation is altered. For example, genome-wide methylene analysis revealed different DNA methylation patterns in colon cancer cells. Related to this, cell death upon exogenous expression of methyltransferases has been reported in eukaryotes. Expression of mouse DNA methyltransferases induces lethality in a fly and a frog (Table 1). The underlying mechanisms that trigger cell death, and the biological significance of the lethality of these heterologous systems remain unclear.

13. Evolutionary genomics of McrBC family suggesting their evolution as mobile elements

As described above, McrBC restricts infection of phages carrying hydroxymethylcytosine and maintains epigenomes through cell death upon entry or
activation of DNA methyltransferase genes.\textsuperscript{1,107,108} The question of the factors that have been important in increasing the frequency of \textit{mcrBC} genes was answered through evolutionary genomics analysis.\textsuperscript{1}

If McrBC homologues show a very narrow distribution, and if this correlates with the distribution of phages with hydroxymethylcytosine, the phage defence hypothesis might be favoured as an explanation for the selective advantage conferred by McrBC. However, comprehensive phylogenetic analysis of McrBC homologues revealed the opposite that they are widely distributed in Bacteria and Archaea.\textsuperscript{1}

Phylogenetic trees of the McrB and McrC homologues showed very similar topologies, suggesting strong co-evolution of these two proteins. Detailed analysis of these trees revealed frequent horizontal transfer of \textit{mcrBC} genes between distantly related genomes and frequent loss from a higher-order taxonomic group. Comparing the frequency of pentanucleotide ‘words’ within \textit{mcrBC} genes with the average word frequency of the entire genome suggested that roughly one-third of \textit{mcrBC} genes showed a significant likelihood of recent horizontal gene transfer from a distantly related group.\textsuperscript{1} This argues against the hypothesis that they are conserved only because of their utility for defence against limited phages or other parasites and favours the hypothesis that they behave as selfish (host-killing) mobile elements. The possibility that the host killing could increase their genetic frequency is discussed in Section 14.

Another feature revealed by the phylogenetic trees is the presence of two diverged subfamilies of McrBC-like systems, one comprising the known McrBCs and the other comprising solely uncharacterized homologues with the McrC-like component defined as the uncharacterized protein family DUF524. Members of these two subfamilies show complementary phylogenetic distribution, which probably reflects some degree of mutual incompatibility.

McrBC family members appear to be quite divergent in sequence. Such diversity might be accompanied by diversity in their target recognition. Indeed, some members of one McrBC subfamily have been shown to be Type II-like, because they cleave a specific sequence when unmethylated.\textsuperscript{120} The presence of two \textit{mcrB} paralogues that have diverged in the N-terminus in one genome (see above) is consistent with their divergence in sequence recognition.

Genomic neighbourhood analysis revealed that \textit{mcrBC} genes are frequently linked to homologues of integrase and transposase genes. Several \textit{mcrBC} homologues clearly occur as an insert into a restriction modification gene complex, which results in their linkage, as discussed above. In addition, several McrBC-like systems have been found on plasmids. These findings also indicate potential mobility of the \textit{mcrBC} unit.\textsuperscript{109}

14. Genetic drive as a consequence of genetic conflicts and cell death

We reviewed several forms of cell death after intragenomic conflicts in which an epigenetic system was involved. McrBC restricts invading epigenetic systems with some specificity through host killing. By this mechanism, it defines an epigenetic status in cooperation with a compatible DNA methyltransferase. The significance of these systems on epigenome integrity is discussed above (Section 12). In addition to these biological roles, the mutually exclusive interaction between genes that program cell death (\textit{mcrBC}) and an invading methyltransferase gene may give host cells an advantage in defence against viral infection when the virus carries a methyltransferase gene. This advantage would increase the frequency of the \textit{mcrBC} genes. Such an effect is referred to as ‘genetic drive’.\textsuperscript{121}

Defence against invasion of genetic elements through cell death has been well studied for multicellular eukaryotic cells such as virus-infected mammalian and plant cells.\textsuperscript{122} Generally, virus genome multiplication and virus particle production are dependent on the host biosynthesis machinery. Progeny virus particles infect other cells (Fig. 6A), so death of an infected cell aborts virus multiplication,
336 Restriction in Epigenetic Conflicts

preventing secondary infection of neighbouring cells (Fig. 6B). Similar processes against virus infection for bacteria are ‘phage exclusion’ or ‘phage abortion’ (Fig. 6B). The underlying molecular mechanisms have been determined in detail in some cases. For example, the prr gene in some E. coli strains senses bacteriophage T4 infection and triggers cell death by cleaving host tRNALys. This relationship between host and virus is analogous to an mcrBC+ cell and a phage carrying a DNA methyltransferase gene (Figs 3B and 6B).

Genes that program the death of their host bacterial cell are expected to increase in frequency because of the abortive effect on viral multiplication. The gene programming suicide of infected cells would survive in uninfected cells because of the induction of death in infected cells. The driving effect of the death gene would be clear when the frequency of cells carrying the death gene is compared with the competitor cells without the death gene after viral infection. This hypothesis needs experimental testing and mathematical justification using evolutionary game theory.

For genes that program host killing upon loss, such as Type II restriction–modification systems (Fig. 3A, Section 5), mathematical justification in the domain of evolutionary game theory has shown that the gene complex that programs cell death increases its frequency in the presence of a competitive genetic element. However, an earlier investigation did not demonstrate their spread. This analysis used a model lacking spatial structure, such as a well-mixed liquid culture, where every cell can potentially interact with every other cell. Our group demonstrated that these genes can increase in frequency from near zero, if a spatial structure is present that allows a cell to preferentially interact with its neighbours. Their increase also depended on the relative cost of the host-killing gene (and its competitor) on the host and on their rate of horizontal transfer.

In phage exclusion (Fig. 6) and post-segregational killing (Fig. 3A), the conflict between different genetic elements within a genome leads to cell death. In the former, the conflict is phage genome vs. death gene, whereas in the latter, it is the invading competitor genetic element versus the death gene. These conflicts are often called ‘intragenomic conflicts’, although ‘genetic conflicts’ may be more appropriate in many prokaryotic cases, where the genes frequently move between genomes.

15. Other cases suggesting conflicts involving epigenetic systems

Other cases of a mutually exclusive relationship between epigenetic systems involving DNases have been reported. An earlier observation with Mrr, another Type IV restriction enzyme, can be interpreted as a similar conflict with a DNA methyltransferase gene leading to lethal chromosome cleavage (Table 1). Mrr cells show SOS induction and poor growth in the presence of the M.HhaI methyltransferase. Mrr weakly restricts phage lambda that has been modified by M.HhaI. However, infection of a phage lambda carrying the mrr gene to a cell with the m.HhaI gene is severely restricted. These results suggest that Mrr also causes cell death in the presence of an incompatible methyltransferase through chromosomal cleavage, although evidence for this cleavage was not reported. Mrr also induces cell death under high-pressure stress, possibly through chromosomal breakage, although whether epigenetic DNA methylation is involved is not known. The mrr gene forms a cassette with mcrBC and the EcoKI (Type I) restriction–modification system in the E. coli genome, suggesting potential cooperation between these elements to define an epigenome status (Section 12, Fig. 5). This linkage is also observed in Xanthomonas, where an mrr homologue is linked with a Type I restriction–modification system.

A unique family of Mrr-like restriction endonucleases was identified recently. One of its members, MspJ, recognizes 5′-mCNNR (R = G/A). Besides 5-methylcytosine, MspJ also recognizes 5-hydroxymethylcytosine but is blocked by 5-glucosylhydroxymethylcytosine. Several other close homologues of MspJ show similar modification-dependent endonuclease activity and display substrate preferences different from MspJ. They may have similar conflict with methyltransferase genes.

McrA, another Type IV restriction enzyme, restricts uptake of DNAs with m5C modifications and is mutually exclusive with M.HpaII. Whether this exclusion occurs through chromosomal cleavage and is accompanied by cell death, as for McrBC, is not known. An mcrA homologue of Burkholderia sp. 383 appears to have inserted with no other linked genes, with a long target duplication similar to other Type I, II, and III restriction systems. In E. coli, the mcrA gene is on the prophage element e14. These observations indicate the potential mobility of mcrA genes, with or without other mobile elements.

The Type IV restriction enzyme GmrSD was found in an E. coli strain and it targets glycosylated hydroxymethylcytosine. Cytosine-containing phage are sensitive to Type II restriction enzymes. Hydroxymethyl-cytosine-containing phage are resistant to Type II restriction enzymes but sensitive to McrBC. Glycosylated hydroxymethyl-cytosine-containing phage are resistant to McrBC but sensitive to GmrSD. GmrSD is inhibited by an internal protein coded by some of the phage. These suggest an
ever-lasting evolutionary arms race between the bacteria and the phage.

Bacteriophage Mu DNA contains unusual deoxynucleoside, alpha-N-(9-beta-D-deoxyribofuranosylpurin-6-yl)glycinamid, specified by the mom gene, which make it resistant to several Type II restriction enzymes and a Type III enzyme.133

The methyl-DNA-specific restriction enzyme DpnI, and the Type II restriction enzyme DpnII have complementary endonuclease activities: DpnI cleaves the modified DNA sequence 5′-Gm6ATC, whereas DpnII cleaves the same recognition sequence only when it is not methylated.134,135 DpnII forms a restriction–modification system with two methyltransferase genes that provide 5′-Gm6ATC modification. Although these complementary endonucleases were isolated from the same Streptococcus pneumoniae species, they do not co-exist in the same genome, probably because of their endonuclease activities.136 They are located at the same locus and share flanking sequence homology,136 suggesting that these restriction cassettes exchange with each other and establish two distinct epigenetic states.136

DpnI is classified as Type IIM restriction enzyme, which targets specific methylated sequence. BslI, BsiI, Glul, and Glal also belong to this class and cleave specific DNA sequence with 5-methylcytosine.137 BslI, BsiI, and Glul are isoschizomers and hydrolyze the DNA sequence 5′-GCNGC, which is methylated in different ways. Glal cleaves the DNA sequence 5′-GmCGC if there are two, three, or four 5-methylcytosines. In some phage DNAs, there is a 5-methylcytosine in the nucleotide sequence 5′-GmCGC, which is methylated by Glal, but Glal displays minimal activity in its cleavage. Therefore, their role in a bacterial cell has remained unclear. Their role may be in conflict with specific methyltransferase genes by cleaving methylated genomic DNA at numerous sites to cell death.

Conflicts that do not cause cell death between restriction–modification systems and anti-restriction systems encoded by bacteriophages and plasmids have also been reported.138 These systems inhibit Type I restriction through different mechanisms, and some can affect methylation activity. For example, Ocr from phage T7 mimics DNA and antagonizes the methyltransferase complex, inhibiting the restriction and modification activities.139,140 Phage T3 encodes an enzyme that degrades S-adenosylmethionine, an essential cofactor for Type I restriction and modification.141 Ral of phage lambda and Lar of the Rac prophage protect infecting DNA from Type I restriction by enhancing methyltransferase activity.142,143 Proteins that can regulate DNA methyltransferase activity represent another factor that can affect epigenome status.

To our knowledge, genetic or epigenetic conflicts specific to Type III restriction–modification systems have not been published so far. However, conflicts described for the other types of restriction systems in this review article might be applicable to this family.

Recently, a novel type of host-specific restriction–modification systems that do not employ methylation was found. Phosphorothioation of DNA by products of specific gene clusters protects DNA against a DNase controlled by the same gene cluster.144,145 Similar or homologous systems are found in many bacteria, but their biological significance remains unclear. No DNases specific to the S-modification have been reported so far.

16. Conclusion and perspective

We have reviewed conflicts between different genetic and epigenetic elements within a genome, focusing on prokaryotic DNases that may cause cell death through chromosomal cleavage. The paradigm we propose is that restriction systems enforce an epigenomic status through cell death. Type II restriction–modification systems cause cell death when methylation is decreased. Host killing by Type I restriction enzymes occurs also when the epigenome status is disturbed by DNA damage and repair under several conditions. Entry or activation of an epigenetic DNA methylation system also causes cell death through chromosomal breakage by methyl-DNA-specific DNases that lead to elimination of cells with an altered epigenome. This process maintains the epigenetic status of the cells, sometimes in cooperation with a linked DNA methyltransferase.

For the death gene, host killing appears to be a strategy that maintains or increases their frequency. For the genome, or a society composed of genes, stimulating cell death upon entry or activation of potentially hazardous genetic or epigenetic systems may be more advantageous than accepting them. The conflicts with them might otherwise decrease fitness, especially when brought about by novel invading genetic or epigenetic elements that have not undergone evolutionary selection in the new environment. Establishment of such a hazardous system in one cell might let it spread in the clonal cell population (Fig. 6A). In this case, the active cell death pathway would be selected. For the genome society, these cell deaths represent a form of resolution of intragenomic conflicts.

These restriction systems provide strong evidence for the presence of conflicts between epigenetic systems. They will serve as simple model systems for
gaining insight into the complex but fascinating epigenetic interactions between genes and genomes.

Acknowledgements: We thank Dr Katsumi Isono for the invitation to write this review article.

Funding

This work was supported by Grants-in-Aid for Scientific Research from Japan Society for the Promotion of Science (JSPS), NEDO, the global COE program ‘Genome Information Big Bang’ from Ministry of Education, Culture, Sports, Science, and Technology (MEXT) to I.K. and the Sasakawa Scientific Research Grant from The Japan Science Society to K.I.

References

1. Fukuda, E., Kaminska, K.H., Bujnicki, J.M. and Kobayashi, l. 2008, Cell death upon epigenetic genome methylation: a novel function of methyl-specific deoxyribonucleases, *Genome Biol.*, 9, R163.
2. Lyko, F., Ramsahoye, B.H., Kashlevsky, H., et al. 1999, Mammalian (cytosine-5) methyltransferases cause genomic DNA methylation and lethality in *Drosophila*, *Nat. Genet.*, 23, 363–6.
3. Kimura, H., Suetake, I. and Tajima, S. 2002, Exogenous expression of mouse Dnmt3 induces apoptosis in *Xenopus* early embryos, *J. Biochem.*, 131, 933–41.
4. Roos, W.P. and Kaina, B. 2006, DNA damage-induced cell death by apoptosis, *Trends Mol. Med.*, 12, 440–50.
5. Warren, R.A. 1980, Modified bases in bacteriophage DNAs, *Annu. Rev. Microbiol.*, 34, 137–58.
6. Serrano-Heras, G., Bravo, A. and Salas, M. 2008, Phage phi29 protein p56 prevents viral DNA replication impairment caused by uracil excision activity of uracil-DNA glycosylase, *Proc. Natl Acad. Sci. USA*, 105, 19044–9.
7. Jones, P.A. and Baylin, S.B. 2002, The fundamental role of epigenetic events in cancer, *Nat. Rev. Genet.*, 3, 415–28.
8. Bird, A. 2002, DNA methylation patterns and epigenetic memory, *Genes Dev.*, 16, 6–21.
9. Esteller, M. 2005, Aberrant DNA methylation as a cancer-inducing mechanism, *Annu. Rev. Pharmacol. Toxicol.*, 45, 629–56.
10. Wion, D. and Casadesus, J. 2006, N6-methyl-adenine: an epigenetic signal for DNA-protein interactions, *Nat. Rev. Microbiol.*, 4, 183–92.
11. Casadesus, J. and Low, D. 2006, Epigenetic gene regulation in the bacterial world, *Microbiol. Mol. Biol. Rev.*, 70, 830–56.
12. Collier, J. 2009, Epigenetic regulation of the bacterial cell cycle, *Curr. Opin. Microbiol.*, 12, 722–9.
13. Marinus, M.G. and Casadesus, J. 2009, Roles of DNA adenine methylation in host-pathogen interactions: mismatch repair, transcriptional regulation, and more, *FEBS Microbiol. Rev.*, 33, 488–503.
14. Wilkins, J.F. 2005, Genomic imprinting and methylation: epigenetic canalization and conflict, *Trends Genet.*, 21, 356–65.
15. Srikhanta, Y.N., Maguire, T.L., Stacey, K.J., Grimmond, S.M. and Jennings, M.P. 2005, The phase-variation: a genetic system controlling coordinated, random switching of expression of multiple genes, *Proc. Natl Acad. Sci. USA*, 102, 5547–51.
16. Li, E., Bestor, T.H. and Jaenisch, R. 1992, Targeted mutation of the DNA methyltransferase gene results in embryonic lethality, *Cell*, 69, 915–26.
17. La Salle, S., Mertineit, C., Taketo, T., Moens, P.B., Bestor, T.H. and Trasler, J.M. 2004, Windows for sex-specific methylation marked by DNA methyltransferase expression profiles in mouse germ cells, *Dev. Biol.*, 268, 403–15.
18. Miura, A., Yonebayashi, S., Watanabe, K., Toyama, T., Shimada, H. and Kakutani, T. 2001, Mobilization of transposons by a mutation abolishing full DNA methylation in *Arabidopsis*, *Nature*, 411, 212–4.
19. Medigue, C., Rouxel, T., Vigier, P., Henaut, A. and Danchin, A. 1991, Evidence for horizontal gene transfer in *Escherichia coli* speciation, *J. Mol. Biol.*, 222, 851–6.
20. Gunthert, U. and Trautner, T.A. 1984, DNA methyltransferases of *Bacillus subtilis* and its bacteriophages, *Curr. Top Microbiol. Immunol.*, 108, 11–22.
21. Bujnicki, J.M. and Radlinska, M. 1999, Molecular evolution of DNA-(cytosine-N4) methyltransferases: evidence for their polyphyletic origin, *Nucleic Acids Res.*, 27, 4501–9.
22. Nobusato, A., Uchiyama, I. and Kobayashi, I. 2000, Diversity of restriction-modification gene homologues in *Helicobacter pylori*, *Gene*, 259, 89–98.
23. Nobusato, A., Uchiyama, I., Ohashi, S. and Kobayashi, I. 2000, Insertion with long target duplication: a mechanism for gene mobility suggested from comparison of two related bacterial genomes, *Gene*, 259, 99–108.
24. Kobayashi, I. 2001, Behavior of restriction-modification systems as selfish mobile elements and their impact on genome evolution, *Nucleic Acids Res.*, 29, 3742–56.
25. Roberts, R.J. REBASE, http://rebase.neb.com/rebase/rebase.html.
26. Pingoud, A., Fuxreiter, M., Pingoud, V. and Wende, W. 2005, Type II restriction endonucleases: structure and mechanism, *Cell Mol. Life Sci.*, 62, 685–707.
27. Roberts, R.J., Belfort, M., Bestor, T., et al. 2003, A nomenclature for restriction enzymes, DNA methyltransferases, homing endonucleases and their genes, *Nucleic Acids Res.*, 31, 1805–12.
28. Orlowski, J. and Bujnicki, J.M. 2008, Structural and evolutionary classification of Type II restriction enzymes based on theoretical and experimental analyses, *Nucleic Acids Res.*, 36, 3552–69.
29. Studier, F.W. and Bandonpadhyay, P.K. 1988, Model for how type I restriction enzymes select cleavage sites in DNA, *Proc. Natl Acad. Sci. USA*, 85, 4677–81.
30. Brammar, W.J., Murray, N.E. and Winton, S. 1999, Restriction of *trp* Bacteriophages by *Escherichia coli* K, *J. Mol. Biol.*, 90, 633–47.
31. Janscak, P., MacWilliams, M.P., Sandmeier, U., Nagaraja, V. and Bickle, T.A. 1999, DNA translocation
blockage, a general mechanism of cleavage site selection by type I restriction enzymes, EMBO J., 18,
2638–47.
32. Stanley, L.K., Seidel, R., van der Scheer, C., Dekker, N.H., Szzelkun, M.D. and Dekker, C. 2006, When a helicase
is not a helicase: dsDNA tracking by the motor protein EcoR124I, EMBO J., 25, 2230–9.
33. Ishikawa, K., Handa, N. and Kobayashi, I. 2009, Cleavage of a model DNA replication fork by a Type I
restriction endonuclease, Nucleic Acids Res., 37, 3531–44.
34. Bickle, T.A. 1993, In: Linn, SM, Lloyod, RS and Roberts, R.J., eds, Nucleases, Cold Spring Harbor
Laboratory Press: New York, pp. 89–109.
35. Dryden, D.T.F., Murray, N.E. and Rao, D.N. 2001, Nucleoside triphosphate-dependent restriction
enzymes, Nucleic Acids Res., 29, 3728–41.
36. Meisel, A., Mackeldanz, P., Bickle, T.A., Kruger, D.H. and Schroeder, C. 1995, Type III restriction endonucleases
translocate DNA in a reaction driven by recognition site-specific ATP hydrolysis, EMBO J., 14, 2958–66.
37. Reich, S., Goss, I., Reuter, M., Rabe, J.P. and Kruger, D.H. 2004, Scanning force microscopy of DNA transloca-
tion by the Type III restriction enzyme EcoP15I, J. Mol. Biol., 341, 337–43.
38. Crampton, N., Yokokawa, M., Dryden, D.T.F., et al. 2007, Fast-scan atomic force microscopy reveals that
the type III restriction enzyme EcoP15I is capable of DNA translocation and looping, Proc. Natl Acad.
Sci. USA, 104, 12755–60.
39. Crampton, N., Roes, S., Dryden, D.T.F., Rao, D.N., Edwardson, J.M. and Henderson, R.M. 2007, DNA
looping and translocation provide an optimal cleavage mechanism for the type III restriction enzymes, EMBO J.,
26, 3815–25.
40. Raghavendra, N.K. and Rao, D.N. 2004, Unidirectional translocation from recognition site and a
necessary interaction with DNA end for cleavage by Type III restriction enzyme, Nucleic Acids Res., 32,
5703–11.
41. Ramanathan, S.P., van Aelst, K., Sears, A., et al. 2009, Type III restriction enzymes communicate in 1D
without looping between their target sites, Proc. Natl Acad. Sci. USA, 106, 1748–53.
42. Kelleher, J.E. and Raleigh, E.A. 1991, A novel activity in Escherichia coli K-12 that directs restriction of DNA
modified at CG dinucleotides, J. Bacteriol., 173, 5220–23.
43. Waite-Rees, PA., Keating, C.J., Moran, L.S., Slatko, B.E., Hornstra, L.J. and Benner, J.S. 1991, Characterization and
expression of the Escherichia coli Mrr restriction system, J. Bacteriol., 173, 5207–19.
44. Mulligan, E.A., Hatchwell, E., McCorkle, S.R. and Dunn, J.J. 2010, Differential binding of Escherichia
coli McrA protein to DNA sequences that contain the dinucleotide m5CpG, Nucleic Acids Res., 38,
1997–2005.
45. Mulligan, E.A. and Dunn, J.J. 2008, Cloning, purification and initial characterization of E. coli McrA, a
putative 5-methylcytosine-specific nuclease, Protein Expr. Purif., 62, 98–103.
46. Kobayashi, I. 2004, In: Pingoud, A., eds, Restriction Endonucleases, Springer-Verlag: Berlin, pp. 19–62.
47. Furuta, Y., Abe, K. and Kobayashi, I. 2010, Genome
comparison and context analysis reveals putative mobile forms of restriction-modification systems and
related rearrangements, Nucleic Acids Res., 38, 2428–43.
48. Ishikawa, K., Watanabe, M., Kuroita, T., et al. 2005, Discovery of a novel restriction endonuclease by genome
comparison and application of a wheat-germ-based cell-free translation assay. Pabl (5’-GTA/ C) from the hyperthermophilic archaeon Pyrococcus abyssi, Nucleic Acids Res., 33, e112.
49. Watanabe, M., Yuzawa, H., Handa, N. and Kobayashi, I. 2006, Hyperthermophilic DNA methyltransferase
M.Pabl from the archaeanon Pyrococcus abyssi, Appl. Environ. Microbiol., 72, 5367–75.
50. Khan, F., Furuta, Y., Kawal, M., et al. 2010, A putative mobile genetic element carrying a novel type II
restriction-modification system (PluTI), Nucleic Acids Res., 38, 3019–30.
51. Miyazono, K., Watanabe, M., Kosinski, J., et al. 2007, Novel protein fold discovered in the Pabl family of
restriction enzymes, Nucleic Acids Res., 35, 1908–18.
52. Sadykov, M., Asami, Y., Niki, H., et al. 2003, Multiplication of a restriction-modification gene complex, Mol. Microbiol., 48, 417–27.
53. Handa, N., Nakayama, Y., Sadykov, M. and Kobayashi, I., 2001, Experimental genome evolution: large-scale
genome rearrangements associated with resistance to replacement of a chromosomal restriction-modifica-
tion gene complex, Mol. Microbiol., 40, 932–40.
54. Kusano, K., Naito, T., Handa, N. and Kobayashi, I., 1995, Restriction-modification systems as genomic parasites
in competition for specific sequences, Proc. Natl Acad. Sci. USA, 92, 11095–99.
55. Nakayama, Y. and Kobayashi, I., 1998, Restriction-modification gene complexes as selfish gene entities: roles of a regulatory system in their establishment, maintenance, and apoptotic mutual exclusion, Proc. Natl Acad. Sci. USA, 95, 6442–7.
56. Tao, T. and Blumenthal, R.M. 1992, Sequence and characterization of pvulIR, the PvuII endonuclease gene, and of pvulIC, its regulatory gene, J. Bacteriol., 174, 3395–8.
57. Som, S. and Friedman, S. 1993, Autogenous regulation of the EcoRII methylase gene at the transcriptional
level: effect of 5-azacytidine, EMBO J., 12, 4297–303.
58. Mruk, I. and Blumenthal, R.M. 2008, Real-time kinetics of restriction-modification gene expression after entry into a new host cell, Nucleic Acids Res., 36, 2581–93.
59. Sawaya, M.R., Zhu, Z., Mersha, F., et al. 2005, Crystal structure of the restriction-modification system
control element C.Bcll and mapping of its binding site, Structure, 13, 1837–47.
60. McGeohan, J.E., Streeter, S.D., Thresh, S.J., Ball, N., Ravelli, R.B. and Kneale, G.G. 2008, Structural analysis of the genetic switch that regulates the expression of
restriction-modification genes, *Nucleic Acids Res.*, **36**, 4778–87.
61. Ball, N., Streeter, S.D., Kneale, G.G. and McGeehan, J.E. 2009, Structure of the restriction-modification controller protein C.Esp1396I, *Acta Crystallogr. D Biol. Crystallogr.*, **65**, 900–5.
62. Naito, T., Kusano, K. and Kobayashi, I. 1995, Selfish behavior of restriction-modification systems, *Science*, **267**, 897–9.
63. Ichige, A. and Kobayashi, I. 2005, Stability of EcoRI restriction-modification enzymes in vivo differentiates the EcoRI restriction-modification system from other postsegregational cell killing systems, *J. Bacteriol.*, **187**, 6612–21.
64. Kobayashi, I. 2004, In: Funnell, B.E. and Phillips, G., eds, *Plasmid Biology*. ASM Press: Washington, pp. 105–44.
65. Ohno, S., Handa, N., Watanabe-Matsui, M., Takahashi, N. and Kobayashi, I. 2008, Maintenance forced by a restriction-modification system can be modulated by a region in its modification enzyme not essential for methyltransferase activity, *J. Bacteriol.*, **190**, 2039–49.
66. Kohanski, M.A., Dwyer, D.J., Hayete, B., Lawrence, C.A. and Collins, J.J. 2007, A common mechanism of cellular death induced by bactericidal antibiotics, *Cell*, **130**, 797–810.
67. Kohanski, M.A., Dwyer, D.J. and Collins, J.J. 2010, How antibiotics kill bacteria: from targets to networks, *Nat. Rev. Microbiol.*, **8**, 423–35.
68. Asakura, Y. and Kobayashi, I. 2009, From damaged genome to cell surface: transcriptome changes during bacterial cell death triggered by loss of a restriction-modification gene complex, *Nucleic Acids Res.*, **37**, 3021–31.
69. Handa, N., Ichige, A., Kusano, K. and Kobayashi, I. 2000, Cellular responses to postsegregational killing by restriction-modification genes, *J. Bacteriol.*, **182**, 2218–29.
70. Takahashi, N., Naito, Y., Handa, N. and Kobayashi, I. 2002, A DNA methyltransferase can protect the genome from postdisturbance attack by a restriction-modification gene complex, *J. Bacteriol.*, **184**, 6100–8.
71. Chandrashekaran, S., Saravanan, M., Radha, D.R. and Nagaraja, V. 2004, Ca(2+)-mediated site-specific DNA cleavage and suppression of promiscuous activity of KpnI restriction endonuclease, *J. Biol. Chem.*, **279**, 49736–40.
72. Chinen, A., Naito, Y., Handa, N. and Kobayashi, I. 2000, Evolution of sequence recognition by restriction-modification enzymes: selective pressure for specificity decrease, *Mol. Biol. Evol.*, **17**, 1610–9.
73. Doronina, V.A. and Murray, N.E. 2001, The proteolytic control of restriction activity in *Escherichia coli* K-12, *Mol. Microbiol.*, **39**, 416–28.
74. Makovets, S., Doronina, V.A. and Murray, N.E. 1999, Regulation of endonuclease activity by proteolysis prevents breakage of unmodified bacterial chromosomes by type I restriction enzymes, *Proc. Natl Acad. Sci. USA*, **96**, 9757–62.
75. Makovets, S., Powell, L.M., Titheradge, A.J., Blakely, C.W. and Murray, N.E. 2004, Is modification sufficient to protect a bacterial chromosome from a resident restriction endonuclease? *Mol. Microbiol.*, **51**, 135–47.
76. Seidel, R., Bloom, J.G., van Noort, J., et al. 2005, Dynamics of initiation, termination and reinitiation of DNA translocation by the motor protein EcoR124I, *EMBO J.*, **24**, 4188–97.
77. Murray, N.E. 2000, Type I restriction systems: sophisticated molecular machines (a legacy of Bertani and Weigle), *Microbiol. Mol. Biol. Rev.*, **64**, 412–34.
78. Cromie, G.A. and Leach, D.R. 2001, Recombinational repair of chromosomal DNA double-strand breaks generated by a restriction endonuclease, *Mol. Microbiol.*, **41**, 873–83.
79. Blakely, G.W. and Murray, N.E. 2006, Control of the endonuclease activity of type I restriction-modification systems is required to maintain chromosome integrity following homologous recombination, *Mol. Microbiol.*, **60**, 883–93.
80. Ivanic-Bacce, I., Vlasic, I., Cogelja-Cajo, G., Brcic-Kostic, K. and Salaj-Smic, E. 2006, Roles of PriA protein and double-strand DNA break repair functions in UV-induced restriction alleviation in *Escherichia coli*, *Genetics*, **174**, 2137–49.
81. Katna, A., Boratynski, R., Furmanek-Blaszk, B., Zolcinska, N. and Sektas, M. 2010, Unbalanced restriction impairs SOS-induced DNA repair effects, *J. Microbiol. Biotechnol.*, **20**, 30–8.
82. Day, R.S. 3rd 1977, UV-induced alleviation of K-specific restriction of bacteriophage lambda, *J. Virol.*, **21**, 1249–51.
83. Efimova, E.P., Delver, E.P. and Belogurov, A.A. 1988, Alleviation of type I restriction in adenine methylase (dam) mutants of *Escherichia coli*, *Mol. Gen. Genet.*, **214**, 313–6.
84. Garcia, L.R. and Molineux, I.J. 1999, Translocation and specific cleavage of bacteriophage T7 DNA in vivo by EcoKI, *Proc. Natl Acad. Sci. USA*, **96**, 12430–5.
85. Handa, N. and Kobayashi, I. 2005, Type III restriction is alleviated by bacteriophage (RecE) homologous recombination function but enhanced by bacterial (RecBCD) function, *J. Bacteriol.*, **187**, 7362–73.
86. Arifuzzaman, M., Maeda, M., Itoh, A., et al. 2006, Large-scale identification of protein–protein interaction of *Escherichia coli* K-12, *Genome Res.*, **16**, 686–91.
87. Firman, K. and Szczelkun, M.D. 2000, Measuring motion on DNA by the type I restriction endonuclease *EcoR124I* using triplex displacement, *EMBO J.*, **19**, 2094–102.
88. van Aelst, K., Toth, J., Ramanathan, S.P., Schwarz, F.W., Seidel, R. and Szczelkun, M.D. 2010, Type III restriction enzymes cleave DNA by long-range interaction between sites in both head-to-head and tail-to-tail inverted repeat, *Proc. Natl Acad. Sci. USA*, **107**, 9123–8.
91. Raleigh, E.A. 1992, Organization and function of the mcrBC genes of *Escherichia coli* K-12, *Mol. Microbiol.*, 6, 1079–86.

92. Raleigh, E.A., Trimarchi, R. and Revel, H. 1989, Genetic and physical mapping of the mcrA (rglA) and mcrB (rglB) loci of *Escherichia coli*, *Genetics*, 122, 279–96.

93. Ross, T.K. and Braymer, H.D. 1987, Localization of a genetic region involved in McrB restriction of 5-methylcytosine-containing DNA in *Escherichia coli* K12, *Mol. Gen. Genet.*, 216, 402–7.

94. Ross, T.K., Achberger, E.C. and Braymer, H.D. 1989, Nucleotide sequence of the mcrBC region of *Escherichia coli* K-12 and evidence for two independent translational initiation sites at the mcrB locus, *J. Bacteriol.*, 171, 1974–81.

95. Dila, D., Sutherland, E., Moran, L., Slatko, B. and Raleigh, E.A. 1990, Genetic and sequence organization of the mcrBC locus of *Escherichia coli* K12, *J. Bacteriol.*, 172, 4888–900.

96. Kruger, T., Grund, C., Wild, C. and Noyer-Weidner, M. 1992, Characterization of the mcrB region of *Escherichia coli* K-12 wild-type and mutant strains, *Gene*, 114, 1–12.

97. Sutherland, E., Coe, L. and Raleigh, E.A. 1992, McrBc: a multisubunit GTP-dependent restriction endonuclease, *J. Mol. Biol.*, 225, 327–48.

98. Beary, T.P., Braymer, H.D. and Achberger, E.C. 1997, Evidence of participation of McrB(S) in McrBC restriction in *Escherichia coli* K-12, *J. Bacteriol.*, 179, 7768–75.

99. Panne, D., Raleigh, E.A. and Bickle, T.A. 1998, McRAs, a modulator peptide for McrBC activity, *EMBO J.*, 17, 5477–83.

100. Gast, F.U., Brinkmann, T., Pieper, U., Kruger, T., Noyer-Weidner, M. and Pingoud, A. 1997, The recognition of methylated DNA by the GTP-dependent restriction endonuclease McrBC resides in the N-terminal domain of McrB, *Biochim. Biophys. Acta*, 1378, 975–82.

101. Pieper, U., Schweitzer, T., Groll, D.H., Gast, F.U. and Pingoud, A. 1999, The GTP-binding domain of McrC: more than just a variation on a common theme? *J. Mol. Biol.*, 292, 547–56.

102. Panne, D., Raleigh, E.A. and Bickle, T.A. 1999, The McrBC endonuclease translates DNA in a reaction dependent on GTP hydrolysis, *J. Mol. Biol.*, 290, 49–60.
