

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

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## 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)<sup>1–3</sup> 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

**Table 1.** Programmed cell death and changes in epigenetic DNA methylation

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; <i>Escherichia coli</i>	52,53,62	
		Inactivation of DNA methyltransferase	Type II modification gene (for <i>ecoRIIM</i> )	<i>Escherichia coli</i>	65	
		Loss of DNA methyltransferase gene	<i>dam</i>	DNA replication initiation defect in <i>Vibrio cholerae</i>	12	
	Through DNA metabolism	Base substitution mutation		<i>ccrM</i>	DNA replication and cell-cycle defect in <i>Caulobacter crescentus</i>	12
				<i>dnmt1</i> (DNA methyltransferase I)	Knockout, in mice embryos	16
		Interstrand crosslink repair	Type I restriction–modification genes ( <i>ecoKI</i> , <i>ecoR124I</i> , in the absence of restriction alleviation); Type II restriction–modification genes ( <i>ecoRIR</i> , <i>ecoRIM</i> )		75,78,79	
Increase	Exogenous expression of DNA methyltransferase		Type II restriction–modification genes ( <i>ecoRIR</i> , <i>ecoRIM</i> )		81	
			<i>mcrBC</i>		1	
			<i>mrr</i>	Indirect evidence	127	
			<i>dnmt1</i> and <i>dnmt3a</i>	Mouse gene in fly	2	
			<i>dnmt3a</i>	Mouse gene in fly; mouse gene in frog	2,3	
		<i>dmnt3b</i>	Mouse gene in frog	3		

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 (<sup>m5</sup>C), N4-methylcytosine (<sup>m4</sup>C), and N6-methyladenine (<sup>m6</sup>A), which are inherited by maintenance methylation after DNA replication; and (iii) non-genetic and non-epigenetic methylation, e.g. O<sup>6</sup>-methylguanine. The non-epigenetic and the non-genetic DNA methylation in O<sup>6</sup>-methylguanine are known to trigger cell death.<sup>4</sup> 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,<sup>5</sup> and dUMP is often incorporated in place of dTMP into some bacteriophage genomes.<sup>6</sup>

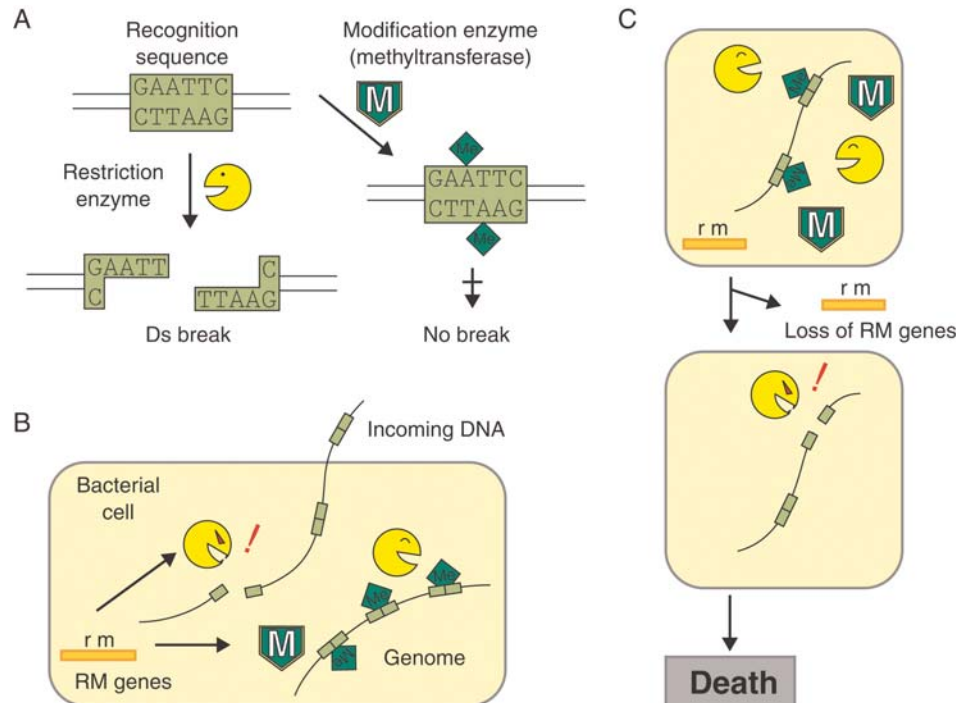
In eukaryotes, epigenetic DNA methylation plays roles in chromatin organization, gene expression, and genome maintenance, and its disturbance is related to human diseases.<sup>7–9</sup> In prokaryotes, it is crucial for processes including cell-cycle regulation, transcriptional regulation, and host-pathogen

interaction.<sup>10–13</sup> It is also involved in silencing selfish genetic elements and other aspects of intragenomic conflicts in eukaryotes<sup>14</sup> and in prokaryotes (this review, see below).

Switching on and off of DNA methyltransferase through phase variation in bacteria can change the entire transcriptome.<sup>15</sup> Experimental alteration of epigenetic DNA methylation systems in prokaryotes can cause a variety of changes.<sup>10,15–18</sup> Horizontal gene transfer between prokaryotic genomes is common,<sup>19</sup> and the DNA methyltransferase genes, in particular, are known to frequently undergo this type of transfer.<sup>20–24</sup> 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.<sup>25</sup> Restriction enzymes are DNA endonucleases that recognize specific DNA sequences and introduce a



**Figure 1.** Action of a Type II restriction–modification system. (A) Restriction enzyme and modification enzyme. The modification enzyme protects the restriction enzyme targets through DNA methylation. (B) Attack on incoming DNA lacking proper methylation. (C) Enforcement of an epigenetic state. After loss of the restriction–modification gene complex or imbalance between restriction and modification, DNA methylation decreases. The restriction enzyme will attack exposed sites, killing the cell. Chromosome breakage may be repaired or may generate a variety of mutated and rearranged genomes, some of which might survive. Ds, double strand; rm, restriction–modification gene complex; RM, restriction modification.

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

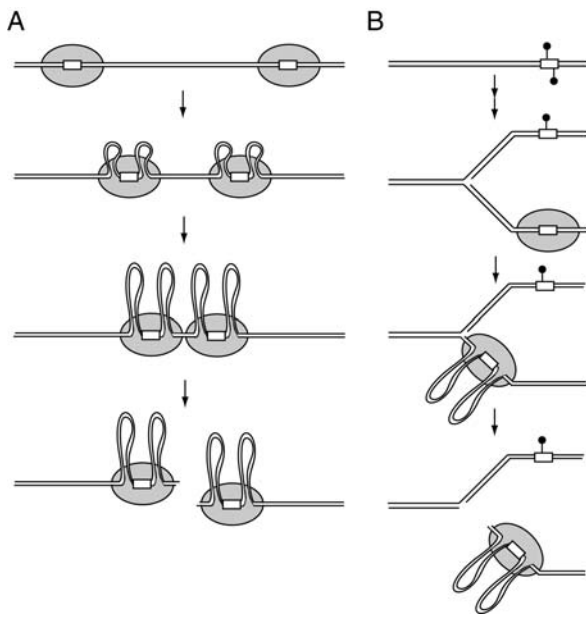
Type II restriction enzymes bind to a recognition sequence and cleave DNA in their vicinity<sup>26</sup> and are frequently used in DNA engineering. For example, EcoRI, BamHI, and PvuII are Type II enzymes. Many variants are classified into subtypes within this type, based on biochemical characteristics.<sup>27</sup> 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.<sup>28</sup>

### 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.<sup>29</sup> This is consistent with an *in vivo* observation.<sup>30</sup> Cleavage can occur also through interaction of the translocating restriction enzyme complex with a Holliday junction,<sup>31</sup> a single-strand gap, a single-strand nick,<sup>32</sup> or a long branch (Fig. 2B; see Section 8).<sup>33</sup>

Type I restriction modification enzymes have two modes of action that are controlled by the



**Figure 2.** Models for Type I restriction enzyme activity. (A) Cleavage upon enzyme collision. After binding to an unmethylated recognition site, a Type I restriction enzyme complex begins pulling dsDNA. DNA is cleaved where two complexes collide. (B) Cleavage at an arrested DNA replication fork. DNA damage leads to aberrant DNA replication initiation, which exposes the unmethylated recognition sites. A Type I restriction enzyme complex begins pulling DNA. DNA is cleaved where the complex reaches an arrested replication fork. Ellipse, Type I restriction enzyme; open square, unmethylated recognition site; filled circle with a bar, methyl group.

methylation state of their recognition sequence.<sup>34</sup> 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.<sup>34</sup>

### 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.<sup>35</sup> The cleavage mechanism is not yet clear, although diverse and sometimes mutually contradictory models have been proposed.<sup>36–41</sup>

### 3.4. Type IV systems

Type IV systems contain a class of enzymes that cleave DNA only when the recognition site is

methylated.<sup>27</sup> In *Escherichia coli*, McrA, McrBC, and Mrr are enzymes in this class that show different restriction spectra.<sup>42</sup> McrBC, the best characterized of this class, is described in detailed below (Section 9). Although McrA and Mrr are believed to be endonucleases, their DNA cleavage activities have not been observed *in vitro*.<sup>43–45</sup>

## 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.<sup>24</sup> 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.<sup>46</sup> 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.<sup>46</sup>

Recently, our group conducted systematic genome comparisons and genome context analysis on fully sequenced prokaryotic genomes to detect restriction–modification-linked genome rearrangements.<sup>47</sup> 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 transposase genes are in classical DNA transposons. Some of these had apparently inserted into a genome with a short target duplication,<sup>47</sup> similar to DNA transposons.

Some of the putative restriction–modification systems in the mobility-related regions are



functional.<sup>48–50</sup> A mobility-based search for novel restriction enzymes detected a previously uncharacterized family of DNA-interacting proteins.<sup>48,50,51</sup> The mobility of McrBC is described in Section 13.

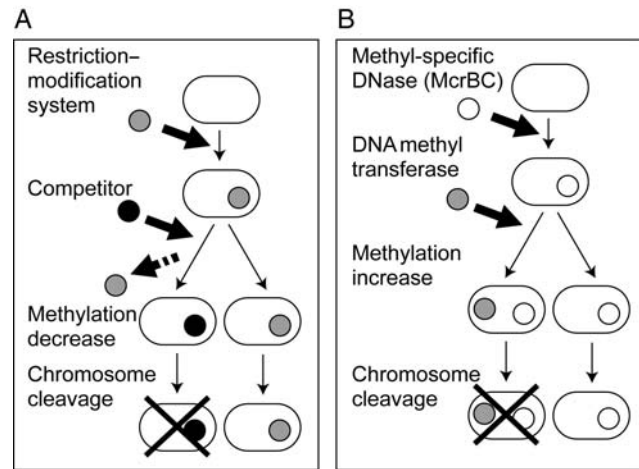
As described in Section 5, Type II restriction–modification systems contribute to their own genetic stability and to the stability of genes linked to them. Their linkage to a mobile element would be mutually beneficial. Restriction–modification systems would stabilize the element, and the mobile element 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<sup>52</sup> and involvement in genome rearrangements<sup>53</sup> under laboratory conditions, as well as by their mutual competition<sup>54</sup> and regulation of gene expression.<sup>46,55</sup> 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.<sup>55–61</sup> This regulation can cause a conflict between two epigenetic systems that is similar to phage exclusion, which is described in Section 6.<sup>55</sup>

### 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).<sup>52,53,62</sup> 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,<sup>63</sup> resulting in undermethylated chromosomes.<sup>53</sup> 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’.<sup>64</sup> Similar host attack can take place after inactivation of the modification enzyme.<sup>65</sup> Host killing forces cells to



**Figure 3.** Host attack by Type II restriction–modification systems and by methyl-specific DNases (McrBC) in competition. (A) Type II systems. When a resident restriction–modification gene complex is replaced by a competitor genetic element, the modification enzyme level decrease exposes newly replicated chromosomal restriction sites to lethal cleavage by the remaining restriction enzymes. Intact genome copies survive in uninfected and unaltered neighbouring clonal cells. (B) McrBC. When a DNA methylation system enters a cell and begins methylating chromosomal recognition sites, McrBC senses the change and triggers cell death by chromosomal cleavage. The intact genome copies survive in uninfected and unaltered neighbouring clonal cells. From Fukuda *et al.*<sup>1</sup>

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.<sup>66,67</sup> Transcriptome analysis during post-segregational death programmed by a Type II restriction–modification system revealed its similarity to death caused by several antibiotics.<sup>68</sup> 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.<sup>69</sup> Host killing by EcoRII restriction–modification system is suppressed by a solitary methyltransferase,

Dcm, which recognizes the same sequence.<sup>70</sup> 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.<sup>71</sup> 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.<sup>54</sup> 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.<sup>54</sup>

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.<sup>72</sup>

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.<sup>70</sup> 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 verified<sup>55</sup> 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.<sup>73–76</sup> Evidence suggests that restriction alleviation is a mechanism for protecting chromosomes from restriction at a newly generated replication fork that produces unmethylated restriction sites.<sup>77</sup> Indeed, chromosome breakage leading to cell death is observed when the restriction alleviation effect is lost for EcoKI<sup>75,78</sup> and EcoR124I<sup>79</sup> (Table 1). Thus, restriction alleviation can be regarded as an indirect sign of a decrease in epigenetic methylation.<sup>80</sup>

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.<sup>80</sup> 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.<sup>80</sup> Through this process, new DNA strands can pair and form unmethylated recognition sites.<sup>80</sup>

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.<sup>78</sup> This mutagenesis generates additional unmethylated recognition sequences for some restriction–modification systems with a methyltransferase protecting DNA with an <sup>m6</sup>A 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.<sup>78</sup> 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).<sup>75</sup>

The lethal effect of mitomycin C, a DNA cross-linker, is enhanced by EcoRI restriction–

modification.<sup>81</sup> 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 EcoR124I.<sup>74,79,82,83</sup> 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.<sup>84,85</sup> 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.<sup>86</sup>

Recently, we demonstrated that the Type I restriction enzyme EcoR124I cleaves model replication forks at their branch point *in vitro*.<sup>33</sup> 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,<sup>84</sup> and the recombination repair of restriction damage observed after a single infection by a phage genome.<sup>85</sup> 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*.<sup>85</sup> However, interaction of Type III and IV enzymes with a branched DNA could be different from that of the Type I enzymes, because their interaction with roadblocks on DNA are different from that of Type I enzymes.<sup>36,87–90</sup>

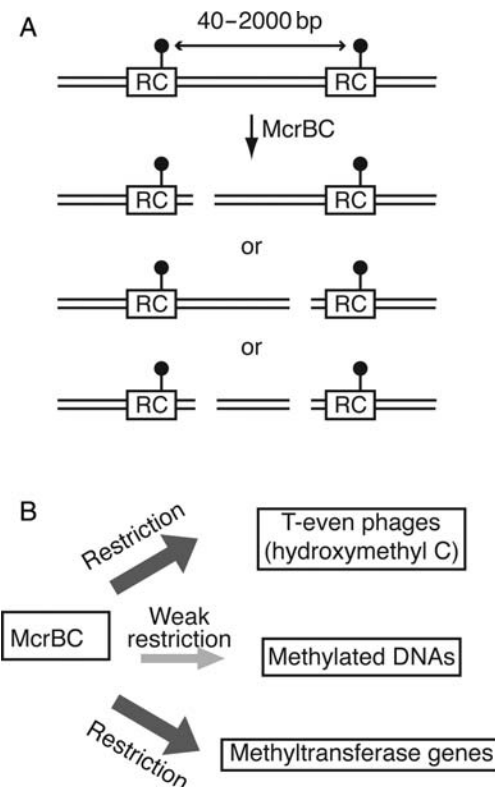
In contrast to cell death in response to a decrease in genome DNA methylation, we discuss cell death in response to its increase in the latter half of this review.

## 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*.<sup>91–94</sup> The *mcrB* gene encodes two different protein forms, McrB<sub>L</sub> (a larger, full-length form) and McrB<sub>S</sub> (a smaller form). These proteins share the same amino acid sequence except for 161 amino acids at the N-terminus that are missing in McrB<sub>S</sub>.<sup>93,95–98</sup> McrB<sub>L</sub> and McrC are sufficient for methyl-DNA cleavage activity,<sup>99</sup> and McrB<sub>S</sub> is involved in activity modulation.<sup>100,101</sup>

McrB<sub>L</sub> is composed of two functional domains. The N-terminal domain specifically binds to a methylated recognition site.<sup>102</sup> The C-terminal domain has GTPase activity and includes three GTP-binding motifs.<sup>103</sup> McrC is the endonuclease subunit with a PD-(D/E)×K motif.<sup>104,105</sup> 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.<sup>28</sup>

The recognition sequence of *E. coli* McrBC is composed of two sites, in the form of R<sup>m</sup>C N<sub>40–2000</sub> R<sup>m</sup>C (where R is A or G).<sup>99,106</sup> 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.<sup>99</sup> 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.<sup>90,99</sup> McrBC binds to the specific recognition site R<sup>m</sup>C through the N-terminal DNA-binding domain of McrB<sub>L</sub>.<sup>102</sup> Double-stranded DNA cleavage occurs



**Figure 4.** Action of McrBC, a methyl-specific DNase. (A) Reaction *in vitro*. McrBC recognizes R<sup>m</sup>C (R = A or G) and cleaves the DNA, usually near a recognition site. Cleavage requires two recognition sites about 40–2000 bp (adapted from Raleigh<sup>91</sup>). (B) Restriction *in vivo*. McrBC strongly restricts T-even phages whose DNA carries hydroxymethyl C in place of C. However, it only weakly restricts plasmids and phages whose DNA has been methylated by a modification enzyme.



through interaction of two McrBC complexes on the DNA.<sup>99</sup> The interaction is facilitated through translocation of the enzyme complexes along the DNA rather than through DNA looping.<sup>90</sup> GTP and Mg<sup>2+</sup> are required cofactors for the cleavage reaction.<sup>99</sup>

Comparison of intragenomic paralogues revealed possible diversification in sequence recognition in McrBC homologues.<sup>1</sup> 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.<sup>1</sup> Alignments of intragenomic McrB homologue pairs revealed amino acid sequence divergence in the N-terminal region that is involved in DNA binding,<sup>102</sup> suggesting evolutionary shifts in DNA sequence specificity.<sup>1</sup> 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 recognized in the restriction of invading bacteriophage genomes carrying hydroxymethylcytosine instead of cytosine (Fig. 4B).<sup>107,108</sup> Bacteriophages carrying this unusual base are rare.<sup>109,110</sup> McrBC may also protect cells against infection by methylated DNAs, such as viral genomes and plasmids, by directly cleaving invading DNA. However, such methylated DNAs are not usually strongly restricted by McrBC (Fig. 4B).<sup>1,42,111</sup> 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–modification 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 methyltransferase 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 epigenetic changes and trigger cell death through chromosomal cleavage. Intact (unmethylated) genomes with *mcrBC* genes would survive in neighbouring clonal cells,<sup>1</sup> 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 chromosomes occurs upon entry or induction of a methyltransferase gene and aborts its establishment or activation.<sup>1</sup> Our genome informatics analysis supported the hypothesis that, during evolution, McrBC has behaved as a mobile element.<sup>1</sup> Therefore, maintenance of McrBC enzymes cannot be explained only

by the restriction of bacteriophages containing hydroxymethylcytosine. 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 carrying a DNA methyltransferase gene cannot be propagated in an *mcrBC*<sup>+</sup> strain of *E. coli* (Fig. 4B).<sup>112</sup> Whether the block to propagation is due to repeated methylation of the introduced DNA and subsequent cleavage<sup>112</sup> or to host genome methylation and its cleavage has not been addressed. Fukuda *et al.*<sup>1</sup> demonstrated that McrBC inhibits establishment of the gene for the DNA methyltransferase *PvuII* (M.PvuII, CAG<sup>m4</sup>CTG) in *E. coli*. Establishment of a plasmid carrying this gene but lacking its recognition sequence was inhibited. This result suggests that the presence of methylated sites on the transferred DNA is not required for McrBC-dependent inhibition,<sup>1</sup> favouring the latter possibility that host genome cleavage accompanied by cell death inhibits the establishment of the methyltransferase 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.<sup>1</sup> Accumulation of huge linear DNAs corresponding to broken chromosomes, and of smaller DNAs of variable size was observed, which likely reflected chromosome degradation. *mcrBC*-dependence strongly suggested that M.PvuII-mediated chromosomal methylation triggered chromosomal cleavage by McrBC, followed by chromosomal degradation. This, in turn, indicates that inhibition of the phage multiplication (restriction) is caused by host death.<sup>1</sup> This kind of conflict between DNA methyltransferase genes carried by bacteriophages and methyl-specific restriction enzymes are biologically relevant because DNA methyltransferase genes are often found in bacteriophage genomes.<sup>25,113–116</sup> 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 chromosomal methylation followed by McrBC-mediated cleavage 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

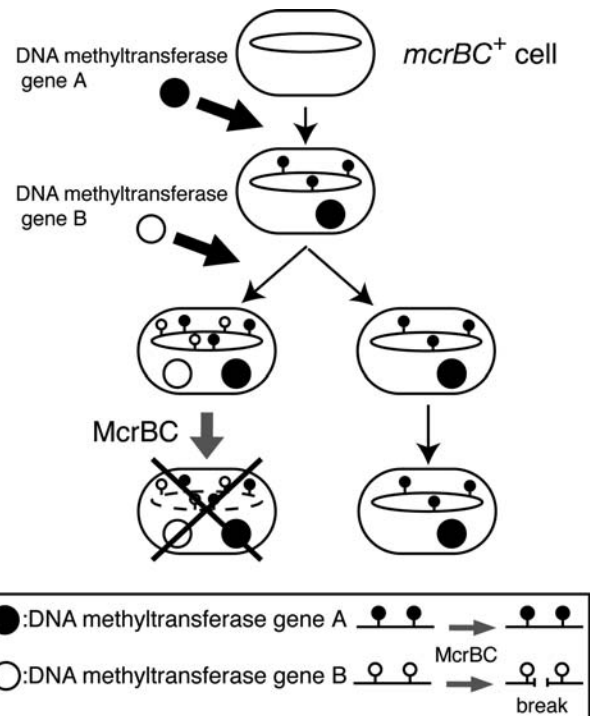
chromosomal methylation leads to McrBC-mediated lethal cleavage.

In addition to M.PvuII, M.SinI (GGW<sup>m5</sup>CC) and M.MspI (m<sup>5</sup>CCGG) causes McrBC-dependent cell death, whereas M.SsoI (C<sup>m5</sup>CNCG) does not. These results are consistent with the R<sup>m5</sup>C sequence specificity of McrBC observed *in vitro*.<sup>99</sup> 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).<sup>54</sup>

## 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,<sup>1,47</sup> as first noted for *E. coli*.<sup>117</sup> 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. m<sup>6</sup>A methylation as opposed to m<sup>4</sup>C and m<sup>5</sup>C 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).



**Figure 5.** Cooperation in epigenome maintenance between McrBC and a DNA methyltransferase. A methyltransferase gene with DNA methylation specificity not subject to McrBC (filled circle) establishes itself in *mcrBC*<sup>+</sup> cells and confers an epigenome state. A methyltransferase gene with specificity subject to McrBC (open circle) cannot establish itself because of host killing through chromosome cleavage by McrBC. Cells with the intact epigenome survive and increase their frequency. Filled circle with a bar, DNA methylation by DNA methyltransferase A; open circle with a bar, DNA methylation by B.

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 methylome analysis revealed different DNA methylation patterns in colon cancer cells.<sup>118,119</sup> 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).<sup>2,3</sup> 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.<sup>1,107,108</sup> The question of the factors that have been important in increasing the frequency of *mcrBC* genes was answered through evolutionary genomics analysis.<sup>1</sup>

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.<sup>1</sup>

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 *mcrBC* genes between distantly related genomes and frequent loss from a higher-order taxonomic group. Comparing the frequency of pentanucleotide 'words' within *mcrBC* genes with the average word frequency of the entire genome suggested that roughly one-third of *mcrBC* genes showed a significant likelihood of recent horizontal gene transfer from a distantly related group.<sup>1</sup> 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.<sup>120</sup> The presence of two *mcrB* paralogues that have diverged in the N-terminus in one genome (see above) is consistent with their divergence in sequence recognition.

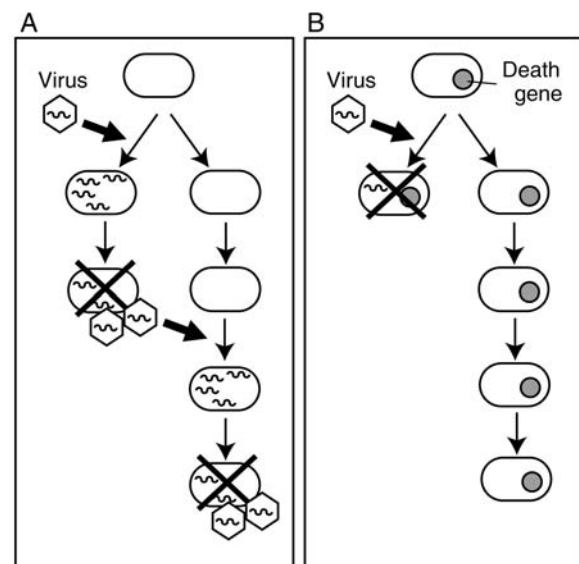
Genomic neighbourhood analysis revealed that *mcrBC* genes are frequently linked to homologues of integrase and transposase genes. Several *mcrBC* homologues clearly occur as an insert into a restriction modification gene complex, which results in their linkage, as discussed above. In addition, several McrBC-like systems have been found on plasmids.

These findings also indicate potential mobility of the *mcrBC* unit.<sup>1</sup>

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

We reviewed several forms of cell death after intra-genomic 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 (*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 *mcrBC* genes. Such an effect is referred to as 'genetic drive'.<sup>121</sup>

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.<sup>122</sup> 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,



**Figure 6.** Defence against viral infection through cell death. (A) Virus infection followed by secondary infection. The virus produces progeny that infect sibling cells. (B) Virus infection to cells that have a gene programming cell death upon the infection. The virus cannot produce progeny. Sibling cells are not infected and survive, together with the death gene.



preventing secondary infection of neighbouring cells (Fig. 6B). Similar processes against virus infection for bacteria are 'phage exclusion' or 'phage abortion' (Fig. 6B).<sup>123</sup> The underlying molecular mechanisms have been determined in detail in some cases. For example, the *prf* gene in some *E. coli* strains senses bacteriophage T4 infection and triggers cell death by cleaving host tRNALys.<sup>124</sup> This relationship between host and virus is analogous to an *mcrBC*<sup>+</sup> 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.<sup>125</sup> However, an earlier investigation did not demonstrate their spread.<sup>126</sup> 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.<sup>125</sup> 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).<sup>127</sup> *Mrr*<sup>+</sup> cells show SOS induction and poor growth in the presence of the *M.Hhal* methyltransferase. *Mrr* weakly restricts phage lambda that has been modified by *M.Hhal*. However, infection of a phage lambda carrying the *mrr* gene to a cell with the *m.hhal* 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,<sup>128</sup> 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).<sup>1</sup> This linkage is also observed in *Xanthomonas*, where an *mrr* homologue is linked with a Type I restriction–modification system.<sup>47</sup>

A unique family of *Mrr*-like restriction endonucleases was identified recently.<sup>129</sup> One of its members, *MspJl*, recognizes 5'-mCNR (R = G/A). Besides 5-methylcytosine, *MspJl* also recognizes 5-hydroxymethylcytosine but is blocked by 5-glucosyl-hydroxymethylcytosine. Several other close homologues of *MspJl* show similar modification-dependent endonuclease activity and display substrate preferences different from *MspJl*. They may have similar conflict with methyltransferase genes.

*McrA*, another Type IV restriction enzyme, restricts uptake of DNAs with <sup>m5</sup>C modifications and is mutually exclusive with *M.Hpal*.<sup>130</sup> 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.<sup>47</sup> In *E. coli*, the *mcrA* gene is on the prophage element e14.<sup>92</sup> 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.<sup>131</sup> 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*.<sup>132</sup> Glycosylated hydroxymethyl-cytosine-containing phage are resistant to *McrBC* but sensitive to *GmrSD*.<sup>131</sup> *GmrSD* is inhibited by an internal protein coded by some of the phage.<sup>131</sup> 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-2'-deoxyribofuranosylpurin-6-yl)glycinamide, specified by the *mom* gene, which make it resistant to several Type II restriction enzymes and a Type III enzyme.<sup>133</sup>

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'-G<sup>m</sup>ATC, whereas DpnII cleaves the same recognition sequence only when it is not methylated.<sup>134,135</sup> DpnII forms a restriction–modification system with two methyltransferase genes that provide 5'-G<sup>m</sup>ATC 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.<sup>136</sup> They are located at the same locus and share flanking sequence homology,<sup>136</sup> suggesting that these restriction cassettes exchange with each other and establish two distinct epigenetic states.<sup>136</sup>

DpnI is classified as Type IIM restriction enzyme, which targets specific methylated sequence. BslI, BlnI, GluI, and Glal also belong to this class and cleave specific DNA sequence with 5-methylcytosine.<sup>137</sup> BslI, BlnI, and GluI are isoschizomers and hydrolyze the DNA sequence 5'-GCNGC, which is methylated in different ways. Glal cleaves the DNA sequence 5'-GCGC if there are two, three, or four 5-methylcytosines. In some phage DNAs, there is a 5-methylcytosine in the nucleotide sequence 5'-GmCGC, a substrate for 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.<sup>138</sup> 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.<sup>139,140</sup> Phage T3 encodes an enzyme that degrades S-adenosylmethionine, an essential cofactor for Type I restriction and modification.<sup>141</sup> Ral of phage lambda and Lar of the Rac prophage protect infecting DNA from Type I restriction by enhancing methyltransferase activity.<sup>142,143</sup> 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.<sup>144,145</sup> 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 intra-genomic 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.

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