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Evolution

Restriction–modification systems as genomic parasites in competition for specific sequences

(selfish gene/methylation/plasmid maintenance/programmed cell death/meiosis)

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ABSTRACT **Restriction–modification (RM) systems are believed to have evolved to protect cells from foreign DNA. However, this hypothesis may not be sufficient to explain the diversity and specificity in sequence recognition, as well as other properties, of these systems. We report that the *Eco*RI restriction endonuclease–modification methylase (rm) gene pair stabilizes plasmids that carry it and that this stabilization is blocked by an RM of the same sequence specificity (*Eco*RI or its isoschizomer, *Rsr* I) but not by an RM of a different specificity (*Pae*R7I) on another plasmid. The *Pae*R7I rm likewise stabilizes plasmids, unless an rm gene pair with identical sequence specificity is present. Our analysis supports the following model for stabilization and incompatibility: the descendants of cells that have lost an rm gene pair expose the recognition sites in their chromosomes to lethal attack by any remaining restriction enzymes unless modification by another RM system of the same specificity protects these sites. Competition for specific sequences among these selfish genes may have generated the great diversity and specificity in sequence recognition among RM systems. Such altruistic suicide strategies, similar to those found in virus-infected cells, may have allowed selfish RM systems to spread by effectively competing with other selfish genes.**

A type II restriction endonuclease makes a double-strand break within or near a specific recognition sequence in duplex DNA. A cognate modification enzyme methylates the recognition sequence to protect it from the cleavage (1, 2). It is widely accepted that the evolution and maintenance of restriction–modification (RM) systems have been driven by the protection from foreign DNA that they afford to cells. The RM systems do protect cells from infection with some viruses by cleaving their DNA (for example, see ref. 3) and are likely to be responsible both for the evolution of antirestriction mechanisms and for the paucity of some restriction sites in certain viruses and plasmids (4).

Recent experimental and theoretical analyses (5, 6), however, seem to us to bring into question the efficacy of virus-mediated selection for RM systems. Defense by RM systems is short-lived because invading viral DNA will occasionally escape restriction and will become modified, thus affording protection from restriction to itself and its descendants (5, 6). Bacteria will more likely develop other, longer-lasting means of resistance to viruses, such as alterations in the receptor required for infection (5, 6). Although RM systems can provide bacteria with advantage when they are invading new habitats full of phages, it is not clear whether such colonization selection is realistic under natural conditions (5, 6).

It is also unclear whether the above "cellular defense" hypothesis can account for the following properties of type II RM systems (1, 2). (*i*) Their individual high specificity and collective wide diversity in the sequence recognition. (*ii*) The tight linkage of cognate restriction and modification genes. (*iii*) No sequence homology among restriction enzyme genes, except for a few pairs, implying their independent evolution. (*iv*) Their ubiquitous occurrence throughout the prokaryotic world, and their virtual absence from the eukaryotic world.

We previously reported that some type II restriction endonuclease gene (r)–modification methylase gene (m) (rm) gene pairs increase stability of plasmids that carry them by causing the death of cells that have lost them (7). Here, we demonstrate competitive exclusion between such selfish rm gene pairs: two RM systems of the same sequence specificity cannot simultaneously force their maintenance on a host. We propose that selfishness and competition underlie the evolution of diversity and specificity in sequence recognition by RM systems.

MATERIALS AND METHODS

Bacteria. *Escherichia coli* strains AB1157 (*rec*$^+$ *racdel*), JC5519 (*racdel recB21 recC22*), JC8679 (*recB21 recC22 sbcA23 rac*$^+$), and DH5 (*recA1 endA1 hsdR17*) have been described (8). DH5 was used for propagation of plasmids.

Plasmids. The plasmids driven by ColE1 replication unit (their rm genotype; antibiotic resistance; source/comments/references) are as follows: pBR322 [none; ampicillin (Amp), tetracycline (Tet); laboratory collection], pMB4 [*Eco*RI r$^+$m$^+$; Amp; F. W. Stahl (9)]; pIK166 (*Eco*RI r$^+$m$^+$; Amp), pIK167 (*Eco*RI r$^-$m$^+$; Amp), pPAORM3.8 (pIK137) [*Pae*R7I r$^+$m$^+$; Amp; T. Gingeras (7, 10)], pTN4 [*Pae*R71 r$^-$m$^+$; Amp (7)], pIK180 (pTZ18R) [none; Amp; K. Mizobuchi (11)], and pIK181 (pTZ18U-*Rsr* I rm) [*Rsr* I r$^+$m$^+$; Amp; A. L. Bari and R. I. Gumport (12)]. The plasmids driven by pSC101ts replication unit are as follows: pHSG415 [none; Amp, chloramphenicol (Cam), kanamycin (Kan) (46)], pIK172 [*Eco*RI r$^+$m$^+$; Amp, Cam (7)], pIK173 (*Eco*RI r$^-$m$^+$; Cam; Amp^s version of pIK172), pIK179 (*Eco*RI r$^-$m$^+$; Cam; Amp^s version of pIK173), pTN9 [*Pae*R7I r$^+$m$^+$; Amp, Cam, Kan (7)], pTN11 [*Pae*R7I r$^-$m$^+$; Amp, Cam, Kan (7)], and pTN18 (*Pae*R7I r$^+$m$^+$; Cam, Kan; Amp^s version of pTN9). The restriction and modification phenotype was verified by plaque assays using λ *cI-71*.

pIK163 was made by replacing a *Bam*HI–*Sal* I fragment of pBR322 with a *Bam*HI–*Sal* I fragment of pMB4. pIK166 was made from pIK163 by deleting a *Bam*HI–*Hin*dIII fragment, which stabilizes plasmids (data not shown). pIK164 was made by cleavage of pIK163 with *Hin*dIII in the r gene, digestion with T4 DNA polymerase, and self-ligation. This should delete 4 bp (bp 526–529) in the r coding region, change the reading frame after amino acid 68, and generate a stop codon (TAG) 25 amino acids downstream. pIK167 was made by deleting a *Bam*HI–*Hin*dIII fragment of pIK164. pIK178 was made by

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Abbreviations: R, restriction; M, modification; r, restriction endonuclease gene; m, modification methylase gene; Amp, ampicillin; Cam, chloramphenicol; Kan, kanamycin.

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inserting a *Bam*HI linker (5′-CCGGATCCGG) into a *Hin*cII site in the *bla* (Amp^r) gene, which confers resistance to Amp. pIK179 was made similarly. pTN18 was made by cleaving the *bla* gene of pTN9 with *Pst* I, removal of 4-bp equivalents with T4 DNA polymerase, and self-ligation.

RESULTS

"Incompatibility" Between Plasmids Carrying rm Gene Pairs of the Same Sequence Specificity. We found that a plasmid is stabilized in an *E. coli rec*$^+$ strain when it carries the *Eco*RI rm genes (Fig. 1). The fraction of host cells that retain the plasmid was measured after prolonged growth in the absence of selection for the plasmid. Our vector, pHSG415, a plasmid driven by the pSC101 replication unit, was more stable when it carried the *Eco*RI rm gene pair than when it carried only the modification component (r^-m^+) or neither component (r^-m^-). This restriction-enzyme-dependent stabilization was also observed in a *recBC* mutant (data not shown) and in a *recBC sbcA* mutant (7). The r^-m^+ version was consistently less stable than the r^-m^- version (Fig. 1; unpublished data), presumably because of the toxic effect of the *Eco*RI methylase.

Stabilization of the plasmid carrying the *Eco*RI rm gene pair was abolished in the presence of a second plasmid carrying the same *Eco*RI rm gene pair and driven by a different replication unit (ColE1 as opposed to pSC101) (Fig. 2*A*). However, the stabilization was not affected by the presence of a second plasmid carrying the *Pae*R7I rm gene pair (10), which recognizes a different sequence (Fig. 2*A*). These and the following experiments used a *recBC sbcA* strain due to the general instability of plasmids in this strain (13).

The *Rsr* I rm and *Eco*RI rm cut and methylate at the same positions of the same target sequence, although the sequences of the genes encoding them are only distantly related (12, 14). Plasmid stabilization by the *Eco*RI rm pair was inhibited by the presence of a second plasmid carrying the *Rsr* I rm pair (Fig. 2*B*). This result is consistent with the hypothesis that the identity of the sequence recognition is the basis of the observed interference between two rm systems.

Interference with plasmid stabilization was also observed when using the *Pae*R7I RM system (Fig. 2*C*). The presence of an rm pair of the same sequence specificity (*Pae*R7I itself) on a second plasmid inhibited the stabilization, whereas an rm pair of a different sequence specificity (*Eco*RI) on a second plasmid did not. The inhibition was observed when the second plasmid carried a mutant version of *Pae*R7I rm, defective in restriction but proficient in modification. This result suggests that the methylation of the recognition sequence is responsible for inhibition of stabilization (Fig. 2*C*). The kinetics of plasmid loss in these experiments are consistent with a simple partition model (15).

Thus, two RM systems of the same sequence specificity cannot simultaneously enjoy stabilization. We call this phenomenon incompatibility between RM systems since it is reminiscent of incompatibility between plasmid replication units. We can say that the *Eco*RI, *Rsr* I, and, presumably, their isoschizomer RMs, which recognize the sequence 5′-GAATTC, form one incompatibility group with respect to plasmid stabilization. The *Pae*R7I and its isoschizomer RMs, which recognize the sequence 5′-CTCGAG, may form another incompatibility group.

Host Killing Model for Stabilization and Incompatibility. These and other observations (7) have led us to hypothesize a

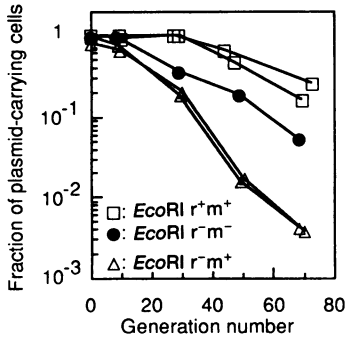

FIG. 1. Stabilization of a plasmid carrying the *Eco*RI rm genes in an *E. coli rec*$^+$ strain. A fresh, single colony of *E. coli rec*$^+$ strain AB1157, carrying pIK172 (r^+m^+) (□), pIK173 (r^-m^+) (△), or pIK174 (r^-m^-) (●), on medium containing Amp was suspended in 5 ml of LB broth (1% tryptone/0.5% yeast extract/1% NaCl). The cells were diluted 1:10^6 in LB broth lacking antibiotics and incubated at 30°C with aeration. Batch culturing was repeated after additional dilutions of 1:10^6. Total cells were counted under a microscope to calculate the generation number. The cells were also spread on LB agar plates to determine viable cells and on agar with ampicillin to determine plasmid-carrying cells. Data are presented as the ratio of plasmid-carrying cells to total viable cells. Amp was used at 50 μg/ml with 200 μg of methicillin per ml.

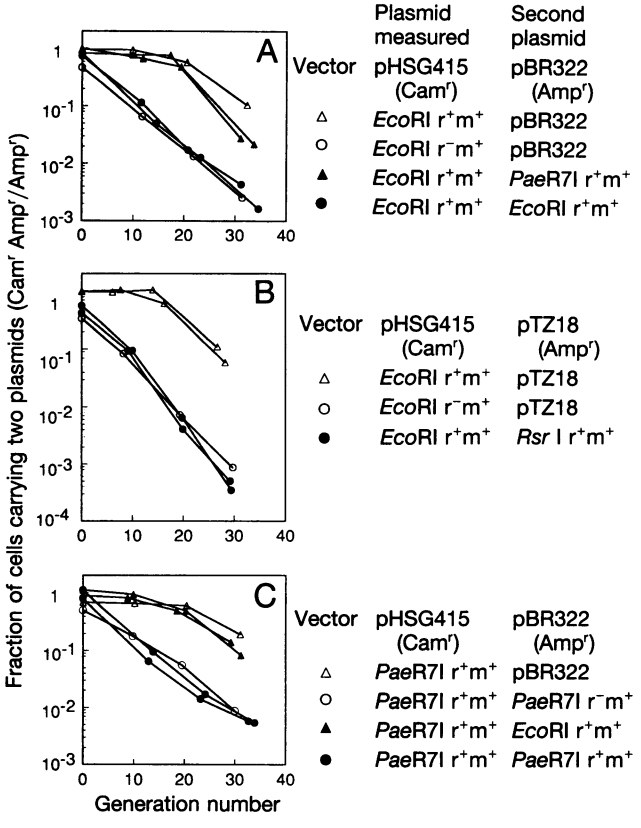

FIG. 2. Stability of an rm plasmid in the presence of a second rm plasmid. (*A*) Stability of a plasmid with the *Eco*RI r^+m^+ (pIK178) or the *Eco*RI r^-m^+ (pIK179) in the presence of a second plasmid with the *Pae*R7I rm (pIK137) or with the *Eco*RI rm (pIK166). *E. coli* strain JC8679 carrying one plasmid with *Eco*RI rm genes (pIK178 or pIK179) and another compatible plasmid (pBR322, pIK137, or pIK166) was grown at 30°C in LB broth with selection for the second plasmid (with Amp) but not for the first plasmid (without Cam). Batch culturing was repeated after dilutions of 1:1000. The ratio of cells carrying both plasmids (Cam^r and Amp^r) to the cells carrying only the second plasmid (Amp^r) was plotted. (*B*) Stability of a plasmid with the *Eco*RI r^+m^+ (pIK178) or the *Eco*RI r^-m^+ (pIK179) in the presence of a second plasmid without rm genes (pIK180) or with the *Rsr* I rm (pIK181). (*C*) Stability of a plasmid with the *Pae*R7I rm (pTN18) in the presence of a second plasmid without rm (pBR322), with the *Eco*RI rm (pIK166), with the *Pae*R7I r^+m^+ (pIK137), or with the *Pae*R7I r^-m^+ (pTN4). Data in *B* and *C* are presented as in *A*. Cam was used at 25 μg/ml.

mechanism for the stabilization by, and incompatibility between, RM systems (Fig. 3). In a cell carrying an rm plasmid, essentially all of the numerous recognition sites on the host chromosome will be modified by the methylation enzyme and protected from the restriction enzyme (Fig. 3*A Upper*). When a cell loses the rm plasmid, its descendants will retain fewer molecules of the modification enzyme after each cell division cycle. Eventually, the modification enzyme will be unable to modify a sufficient number of recognition sites in the chromosome. At this point any remaining restriction enzyme molecules will cut the chromosome at one of these unmodified sites, killing the cell (Fig. 3*A Lower*). The net result is selection for cells carrying the RM plasmid.

This hypothesis explains why stabilization depends on restriction enzyme function (Figs. 1 and 2). This model also explains the observed incompatibility between two RM systems of the same sequence specificity (Fig. 2). The host killing by an rm gene pair will not work when a second rm gene pair of the same sequence specificity (identical, isoschizomer, or neoschizomer system) methylates the recognition sites and protects them from attack by the first restriction enzyme (Fig. 3*C*). On the contrary, the host killing will occur when the second RM system methylates a different recognition sequence (Fig. 3*B*).

Host Killing After Blockage of Replication of an *Eco*RI rm Plasmid and Its Suppression by a Second RM System of the Same Sequence Specificity. To test our hypothesis, we created a situation in which plasmid loss is forced in a majority of cells. We had introduced the *Eco*RI rm gene pair into a temperature-sensitive replicon (pHSG415). The blockage of replication of the rm plasmid was achieved by temperature shift. After a lag period, the shift prevented an increase in the number of viable, plasmid-carrying cells (Fig. 4 *A* and *B*), confirming that replication was inhibited. In cells carrying the plasmid with the *Eco*RI rm gene pair, the shift caused a reduction in the number of viable cells relative to the culture carrying the restriction-defective plasmid (Fig. 4 *A* and *B*). Total cell counts were also reduced when both rm components were present, relative to cell counts when only the modification component was present.

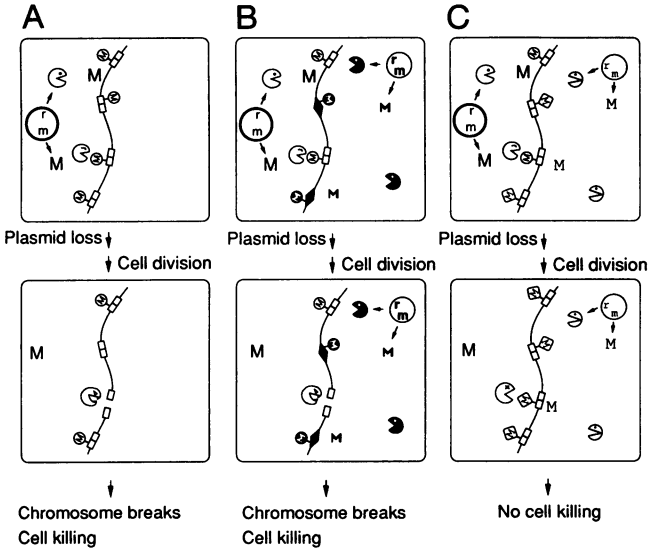

FIG. 3. A host-killing hypothesis for the stable maintenance of plasmids carrying an rm gene pair. (*A*) One plasmid with an rm gene pair. Loss of the plasmid leads to dilution of the modification enzyme and cell killing. (*B*) Two plasmids, with rm gene pairs of different sequence specificity. Loss of one of the plasmids leads to cell killing, as in *A*, independent of the other plasmids. (*C*) Two plasmids with an rm gene pair of the same sequence specificity. Loss of one of the plasmids does not lead to cell killing because the other RM system modifies the recognition sites on the host chromosome.

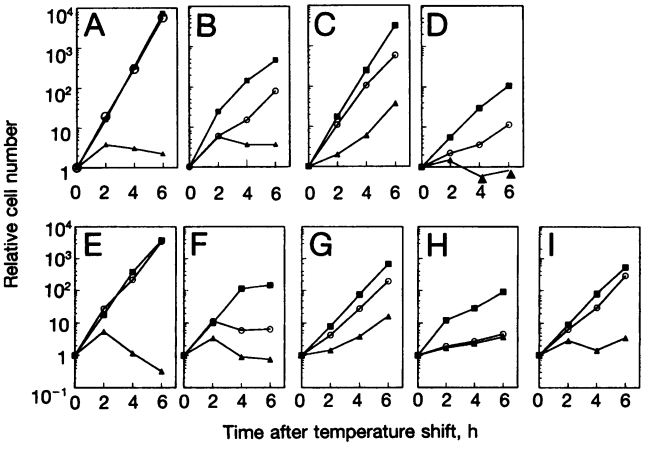

FIG. 4. Cell killing by the *Eco*RI or *Pae*R7I rm gene pairs and its suppression by an rm gene pair with identical specificity. JC8679 cells were aerated at 30°C in broth containing the appropriate selective antibiotics (Amp in *A, B, E,* and *F*; Cam and Amp in *C, D, G, H,* and *I*) until the OD_{660} of the culture reached 0.3. The antibiotics selective for the plasmid of interest (Amp in *A, B, E,* and *F*; Cam in *C, D, G, H,* and *I*) were then removed, and the temperature was shifted to 42°C. The cells were diluted whenever the OD_{660} of the culture reached 0.3. Total cells were counted by using a microscope. The number of viable cells is defined as the number of colonies growing on LB agar plates without antibiotics in *A, B, E,* and *F* or with Amp in *C, D, G, H,* and *I* at 30°C. The number of cells carrying the plasmid of interest was determined by plating on medium containing Amp in *A, B, E,* and *F* or Cam and Amp in *C, D, G, H,* and *I*. JC8679 cells contained the following plasmid(s): pIK173 (*Eco*RI r^-m^+) (*A*); pIK172 (*Eco*RI r^+m^+) (*B*); pIK178 and pIK166 (*Eco*RI r^+m^+ with pBR322::*Eco*RI r^+m^+) (*C*); pIK178 and pIK137 (*Eco*RI r^+m^+ with pBR322::*Pae*R7I r^+m^+) (*D*); pTN11 (*Pae*R7I r^-m^+) (*E*); pTN9 (*Pae*R7I r^+m^+) (*F*); pTN18 and pIK137 (*Pae*R7I r^+m^+ with pBR322::*Pae*R7I r^+m^+) (*G*); pTN18 and pIK166 (*Pae*R7I r^+m^+ with pBR322::*Eco*RI r^+m^+) (*H*); and pTN4 (*Pae*R7I r^+m^+ with pBR322::*Pae*R7I r^-m^+) (*I*). ■, Total cells; ○, viable cells; and ▲, plasmid-carrying cells.

We used pulsed-field gel electrophoresis to analyze the chromosomes of cells used in the above experiments (Fig. 5). With the *Eco*RI rm plasmid, chromosome degradation was detectable 2 h after the blockage of plasmid replication and became more extensive 4 h and 6 h later. No degradation was detectable with the plasmid carrying only the modification component. The observed degradation of the chromosomes coincided with the change in the viable cell counts (Fig. 4 *A* and *B*).

The effect of plasmid loss on cell viability was assessed in the presence of incompatible RM systems by using the forced plasmid loss protocol. Cell killing by *Eco*RI rm was suppressed by the presence of a second plasmid carrying *Eco*RI rm (Fig. 4*C*) but not by the presence of a second plasmid carrying the *Pae*R7I rm (Fig. 4*D*).

The same results were obtained when replication of a plasmid carrying the *Pae*R7I rm was blocked (Fig. 4 *E–H*). The host killing (Fig. 4*F*) was not seen with the r^- version (Fig. 4*E*) (7). The killing was suppressed by a second plasmid carrying the *Pae*R7I rm system (Fig. 4*G*) but not by a second plasmid carrying the *Eco*RI rm system (Fig. 4*H*). The suppression of killing was also seen when a second plasmid carried the *Pae*R7I m gene but not the r gene (Fig. 4*I*). This suggests that the methylase is responsible for the incompatibility, as envisioned in our model (Fig. 3*C*).

DISCUSSION

These and previous results (7) strongly support our host-killing model for the stabilization by rm gene pairs and the incompatibility between rm gene pairs (Fig. 3). The trick of the

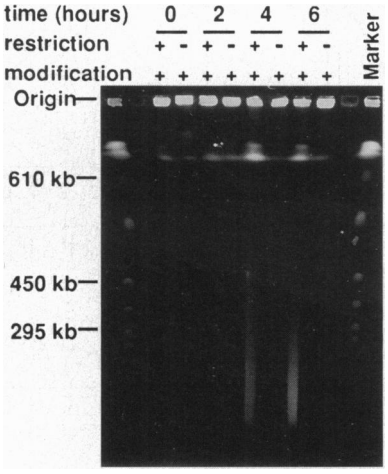

FIG. 5. Degradation of chromosomal DNA accompanies cell killing by the *Eco*RI rm gene pair. Plasmid replication was blocked by shifting cultures of *E. coli* JC8679 carrying pIK173 (*Eco*RI r^-m^+) or pIK172 (*Eco*RI r^+m^+) from 30°C to 42°C. DNA was prepared and subjected to pulsed-field gel electrophoresis (16). The DNA was electrophoresed at 15°C in 45 mM Tris base/45 mM boric acid/1.25 mM EDTA at 165 V with a pulse time of 50 s for 24 h by using hexagonal electrodes in a Pharmacia/LKB apparatus. Marker lanes contain *Saccharomyces cerevisiae* chromosomes.

postsegregational killing by the RM systems lies in the presence of a huge number of the recognition sites in the host chromosome. Its survival will need protection of most or essentially all of them by the methylase, but its death needs effective cleavage of only one of them by the restriction enzyme. It is not necessary to assume differential stability of the restriction enzyme and the methylase. The restriction enzyme and the methylase cannot be simply regarded as a killer and an anti-killer, respectively, because methylases may exert toxic effects on the host cell (Fig. 1) (17).

Our results imply that these rm gene units are selfish gene entities that force their maintenance on the host. These selfish genes "compete" for specific sequences along the host genome. We propose that such selfish behavior underlies the evolution, as well as the maintenance, of type II rm gene units. We will briefly discuss other forms of cell death programmed by selfish genes before we further develop this "selfish gene" hypothesis.

Cell Death Programmed by Selfish Genes. *Postsegregational killing in plasmids.* The host-killing mechanism for maintenance we describe here is similar to the postsegregational killing mechanisms of plasmids such as F (18, 19). These involve a killer and an anti-killer gene pair. Plasmid loss leads to imbalance of the products of the gene pair and cell killing through various mechanisms (20). Our finding was with rm genes carried on plasmids: the *Eco*RI rm on pMB4 (9) and the *Pae*R7I rm on pMG7 (21). The frequency of execution of host-killing by these mechanisms could be very low in the natural situation due to other mechanisms that aid in maintenance of the plasmid (22). pMB4 carries two additional systems for stable maintenance, colicin E1 (22) and site-specific recombination (13).

Some rm genes, such as those in *Bacillus*, are on the chromosome (23). The same cell killing mechanism for stable maintenance should operate in the case of chromosomal rm genes when they are either replaced by a homologous transforming DNA or somehow switched off. Thus, there should be no essential difference in the self-stabilization by host killing whether rm genes are on chromosomes or on plasmids.

Selfish genes causing meiotic drive. Selfish genes in eukaryotic meiosis cause meiotic drive, or preferential transmission of their own allele over the other alleles (24). The action of maternal-effect selfish genes in postfertilization killing, one mechanism of meiotic drive, appears similar to that of the rm genes described here: loss of a selfish gene leads to killing of the progeny by its product (25–29).

Altruistic cell death upon virus infection. In certain cases of viral infection of bacteria or multicellular organisms, an infected cell will commit suicide (20, 30). The driving force in the evolution of such cell death is believed to be prevention of secondary infection by the progeny virus from the infected cell of the neighboring cells, which are very likely of identical genotype. This behavior can be called altruistic in the sense used in the study of social behavior (31). Some of the genes responsible for this cell killing in bacteria are on a prophage (32, 33) and may be regarded as selfish genes. In such cases, the killing may be regarded as altruistic self-defense of these selfish genes.

Selfish-Gene Hypothesis for the Evolution and Maintenance of RM Systems. Our selfish gene hypothesis proposes that altruistic host killing by selfish type II rm gene units underlies their evolution and maintenance in the sense detailed below.

Diversity and specificity in sequence recognition. The individual high specificity combined with the collective wide diversity in the sequence recognition of RM systems is relevant to our demonstration that two rm systems that recognize the same sequence are mutually exclusive in their stabilization by host killing. 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.

Independent evolution of restriction enzymes, as inferred from the virtual absence of their sequence homology, can be understood, at least partly, as specialization by diversifying selection at very early periods. Having a different sequence for each system, a requirement for success by our model, serves to isolate different RM systems in separate niches and ensures their independent evolution. The existence of homologous isoschizomers, such as *Eco*RI and *Rsr* I, is explained as recent divergence and horizontal transfer (see below).

Extrapolation of our competition experiments (Fig. 3) suggests selective pressure for shortening the recognition sequence of an RM system. We hypothesize that their recognition sequences became shorter and shorter during evolution. The diversification of recognition sequences driven by competitive exclusion presumably took place among the siblings from the same parental RM system during this process.

Widespread occurrence. The spreading of selfish genes that kill the host cell is not as paradoxical as it appears. Virulent viruses, such as bacteriophage T4, represent one class of example of such parasites. The host cells serve as their prey. The spreading of meiotic-drive selfish genes similar to the rm genes (see above) is predicted to take place even in the absence of any selective advantage and to be favored both when the populations are divided into small groups and when there is competition for resources between siblings (25, 26, 29). These conditions seem applicable to natural populations of bacteria. There is one theoretical treatment of plasmid-based, postsegregational killing systems (34). That work predicted that the spreading of a postsegregational killing mechanism will be favored only under limited conditions. It took into account the sibling-competition effect but not the small-subgroup effect, as the author admitted (34).

We previously observed this type of competition: a plasmid carrying an rm gene pair cannot be easily displaced by a second plasmid with the same replication unit (and without the recognition sequence) (7). The incoming plasmid was success-

ful in displacing the rm plasmid in the cell, but the resulting cell and the invading plasmid in it failed to survive because of the killing programmed by the rm plasmid (7). This situation is similar to the altruistic suicide for self-defense programmed by selfish genes upon virus infection (see above). A plasmid with an RM system would have an advantage in its competition with its immediate ancestor that lacks the rm system or that has a weaker version. These arguments should apply to replacement of chromosomal rm genes by homologous genes. This is analogous to selection for arms used in battles between animals. It is desirable to formally develop a mathematical model.

Mobility. The selfish gene concept for the rm gene pairs implies a degree of independence from the rest of the genome, which is in keeping with rm gene mobility. The rm genes on conjugative plasmids are mobile, and those on the chromosomes may be mobile by transformation, transduction, or other means. It appears that some rm gene pairs were added to the genome by horizontal transfer (1, 9, 12, 14, 35, 36). This potential mobility is consistent with the tight linkage of r and m genes (1).

Anti-RM systems of bacteria. Our selfish gene hypothesis suggests that bacteria may have evolved anti-RM systems as their viruses have done. We can think of four possible anti-RM mechanisms: (*i*) methyl-specific restriction systems (37, 38); (*ii*) methylation by bacterial solo methylases (39), which may protect bacterial chromosomes from postsegregational cleavage by certain RM systems; (*iii*) homologous recombination (40); and (*iv*) ligation with DNA ligase (41).

Origin of restriction enzymes. Homing endonucleases (mobile introns and inteins) introduce a double-strand break into a long, specific DNA sequence unless the sequence contains their own gene (42). By analogy with the present work, this mechanism may be able to force their stable maintenance on the host by cutting the chromosome when their gene is removed by precise excision.

Many DNA transposons leave a double-strand break at their site on the genome during transposition, and some of them show preference of insertion sites (43, 44). This double-strand breakage could serve the purpose of altruistic cell death.

We hypothesize that selfish endonucleases of this sort, which can kill the host by chromosomal breakage at specific sites under conditions that threaten their survival, such as invasion of competing selfish genes (altruistic cell death strategy), may be the ancestors of the restriction enzymes. These endonucleases presumably came to form a pair with a selfish methylase from one of the few families. We hypothesize that after diversification and shortening of the recognition sequences that was driven by competitive exclusion among their descendants, some of them came to attack viral DNAs by direct cleavage.

Absence from the eukaryotes. Our last mystery concerning RM systems is their absence from the eukaryotic world. We propose that meiosis or, more precisely, meiotic recombination through the double-strand-break repair mechanism is the eukaryotic equivalent of the RM systems (ref. 45; I.K., unpublished data).

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