tained from the indicated sources: B220 (6B2), CD3 (2C11), T cell receptor αβ (H57), T cell receptor γδ (GL3), T200, and 8C5 from Pharmingen; Thy1.1 (M5/49) from Dupont Biotechnology Systems; CD4, CD5, and CD8 from Becton-Dickinson; μ and κ from Southern Biotechnology Associates; J11d, PgP-1 (or CD44), L-PAM, MEL-14, MAC-1, and F4/80 from American Type Culture Collection.
14. Tumor cells were passaged serially in syngeneic mice or in culture in RPMI 1640 supplemented with 10% fetal bovine serum, 4 mM glutamine, penicillin (50 U/ml), streptomycin (50 μg/ml), 50 μM 2-mercaptoethanol, and 1% interleukin-2–conditioned medium obtained from a transfected cell line [H. Karasuyama and F. Melchers, *Eur. J. Immunol.* **18**, 97 (1988)]. To assess the inhibitory effect of antisense CKII oligonucleotides on the growth of the tumor cells, we used a pair of oligonucleotides directed against CKIIα and CKIIβ transcripts that have been shown to inhibit the proliferation of fibroblasts induced by epidermal growth factor [R. Pepperkok, P. Lorenz, R. Jakobi, W. Ansorge, W. Pyerin, *Exp. Cell Res.* **197**, 245 (1991)]. Oligonucleotides (final concentration, 100 μg/ml) were added to 10^5 cells plated in quadruplicate in 100 μl of medium prepared with heat-inactivated serum to improve oligonucleotide stability. After 20 hours of culture, [^{3}H]thymidine (1 μCi per well) was added, and after a further 4 hours, the cells were harvested and the incorporation of [^{3}H]thymidine into DNA (mean ± SD) was determined by scintillation counting.
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Selfish Behavior of Restriction-Modification Systems

Taku Naito, Kohji Kusano, Ichizo Kobayashi*

Plasmids carrying gene pairs encoding type II DNA restriction endonucleases and their cognate modification enzymes were shown to have increased stability in *Escherichia coli*. The descendants of cells that had lost these genes appeared unable to modify a sufficient number of recognition sites in their chromosomes to protect them from lethal attack by the remaining restriction enzyme molecules. The capacity of these genes to act as a selfish symbiont is likely to have contributed to the evolution of restriction-modification gene pairs.

Type II restriction endonucleases introduce double-strand breaks at specific short recognition sequences in duplex DNA provided that the sequences have not been modified by cognate methylases (*1*). Genes that encode cognate restriction-modification (rm) enzymes are normally tightly linked. The conventional hypothesis is that the rm gene pairs evolved under selection pressure to protect cells from infection by foreign (for example, viral) DNA. But it is not clear whether such a "cellular defense" hypothesis explains the extreme diversity and the exceptionally high specificity in their sequence recognition. This hypothesis cannot readily explain the evolution of the "rare cutter" restriction enzymes whose long recognition sequences (~8 base pairs) are unlikely to be present in many bacterial viruses. We present evidence for an alternative hypothesis that the evolution of some rm gene pairs has been driven by their behavior as "selfish" genetic units.

During an analysis of the role of DNA double-strand breaks in homologous recombination in *E. coli* (*2*), we observed that a plasmid carrying the rm genes of Pae R7 could not be readily displaced by a second plasmid devoid of the Pae R7 recognition sequence 5′-CTCGAG-3′. In the initial experiments, the resident r^+m^+ plasmid pIK137 [pPAORM3.8 in (*3*)] and the introduced plasmid pIK52 were incompatible, because both plasmids are driven by a ColE1 replicon. Plasmid pIK52 showed a much lower transformation efficiency when the *E. coli* host carried pIK137 as compared to pBR322, a plasmid without the r^+m^+ genes (Table 1). In addition, the majority of the colonies transformed with pIK52 DNA (42 of 52) were small, and all 24 of the transformants examined retained the resident r^+m^+ plasmid as well as the introduced plasmid (Fig. 1A). When the resident plasmid was an r^-m^+ version of pIK137 (pTN4), the transformation efficiency was not reduced, none of the 300 transformed colonies was small, and none of the 12 transformants examined retained a visible amount of the initially resident plasmid (Fig. 1B). These results suggested that restriction was responsible for the stable retention of the r^+m^+ plasmid.

When replication of the introduced and resident plasmids was driven from different compatible replicons, the presence of an r^+ gene as well as an m^+ gene in the resident plasmid did not influence the transformation efficiency (Table 1). Thus, the low transformation efficiency of pIK52 in the initial experiment was likely due to the resident pIK137 plasmid's enhanced stability, conferred by the rm genes.

To determine whether the r^+m^+ genes could stabilize a plasmid in the absence of a challenge by an incompatible plasmid, we inserted wild-type (r^+m^+) and mutant (r^-m^+) alleles of the Pae R7 rm genes into two different plasmid vectors, one (pBR322) driven by a ColE1 replicon, the other (pHSG415) driven by a pSC101ts replicon (*4*). The insertions into pBR322 were large, and the r^-m^+ version was less stable than pBR322. Nevertheless, the r^+m^+ version was found to be considerably more stable than even pBR322 itself (Fig. 2A). The r gene also had a pronounced stabilizing effect on pHSG415 (Fig. 2B). This pattern was reproduced with another type II rm gene pair, Eco RI (Fig. 2C).

On the basis of these results, we postulated the following mechanism to explain the plasmid stabilization. With successive generations, the descendants of a cell that has lost its r^+m^+ plasmid will contain fewer and fewer molecules of modification en-

Table 1. Resistance of a Pae R7 r^+m^+ plasmid to displacement. *Escherichia coli* strain JC8679 (*7*) carrying the indicated plasmids were transformed by electroporation (*2*) with 500 ng of pIK52 (*9*) or 0.5 ng of pIK134 (*10*). Transformants were selected on LB agar supplemented with kanamycin (50 μg/ml) or chloramphenicol (25 μg/ml), respectively.

Resident plasmid	Transformation efficiency	
	pIK52 (r^-m^-)* (incompatible)	pIK134 (r^-m^-) (compatible)
pBR322 (r^-m^-)	1	1
pIK137 (r^+m^+) (*3*)	0.11	2.3
pTN4 (r^-m^+) (*8*)	4.0	1.8
None	52	2.1

*Average of two experiments; differences between the values obtained were no greater than 10%.

Department of Molecular Biology, Institute of Medical Science, University of Tokyo, Shiroganedai, Tokyo 108, Japan.

*To whom correspondence should be addressed. E-mail: ikobaya@hgc.ims.u-tokyo.ac.jp

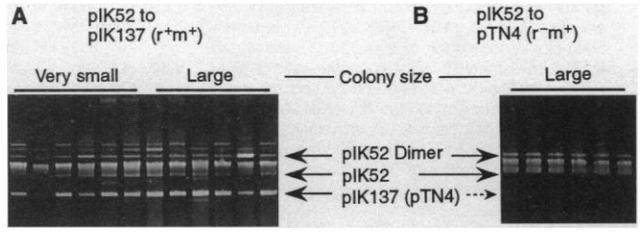

Fig. 1. Persistence of the rm plasmid in transformants. DNA was isolated from the transformants listed in Table 1. (**A**) Introduction of pIK52 into *E. coli* carrying pIK137. (**B**) Introduction of pIK52 into *E. coli* carrying pTN4. The band between the pIK52 monomer and dimer is chromosomal DNA.

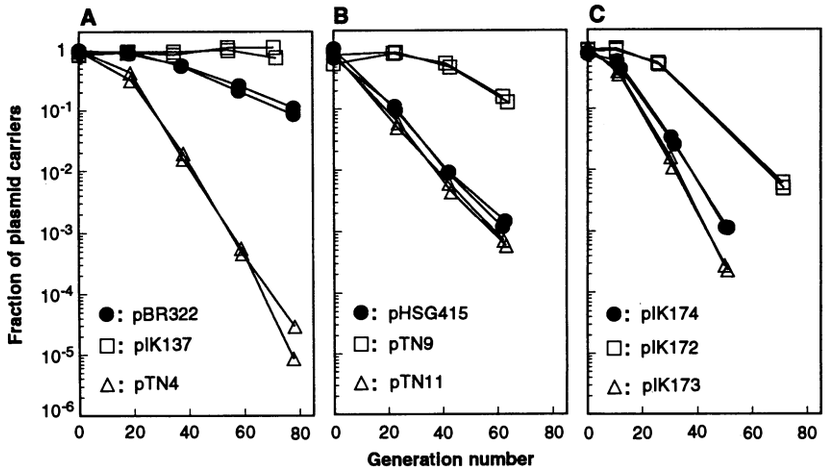

Fig. 2. Stability of rm plasmids in the absence of selection. The fraction of cells carrying the plasmids was measured on LB agar plates supplemented with ampicillin (50 μg/ml) and methicillin (200 μg/ml) by a repeated batch culture method (10^6-fold dilution and overnight incubation) (*11*). (**A**) *Escherichia coli* carrying pBR322, pIK137 (r^+m^+), or pTN4 (r^-m^+) at 37°C. (**B**) *Escherichia coli* carrying pHSG415, pTN9 (r^+m^+), or pTN11 (r^-m^+) (*4*) at 30°C, a temperature permissive for their pSC101ts replicon. (**C**) *E. coli* carrying pIK174 (r^-m^-), pIK172 (r^+m^+), or pIK173 (r^-m^+) (*12*) at 30°C.

zyme. Eventually their capacity to modify the many sites needed to protect the chromosome from the remaining restriction enzyme may be inadequate. The DNA at unmodified sites will then be cleaved and the cells killed. The net result is the stable maintenance of the plasmid genes in the viable cell population. This hypothesis explains why most of the transformant colonies in the initial experiments were very small: The incoming plasmid did not fail to displace the resident plasmid; rather, the cells in which displacement occurred had failed to survive. The small colonies presumably included many dead cells.

To test this hypothesis, we created a condition in which plasmid loss could be thermally induced. We used the r^+m^+ and r^-m^+ Pae R7 genes cloned in the thermosensitive replicon pHSG415. The block to r^+m^+ plasmid replication as the result of a temperature shift had no effect on the viability of cells carrying the r^-m^+ plasmid (Fig. 3A). However, in the case of the r^+m^+ plasmid, the shift stopped the increase in the viable cell count at about the same time as the number of plasmid-carriers stopped increasing, which suggested that the rm enzyme dilution rapidly created a hazardous situation for the host (Fig. 3B). These results indicate that cleavage of unmodified Pae R7 sites by the Pae R7 restriction enzyme is responsible for the killing. The killing was incomplete, presumably be-

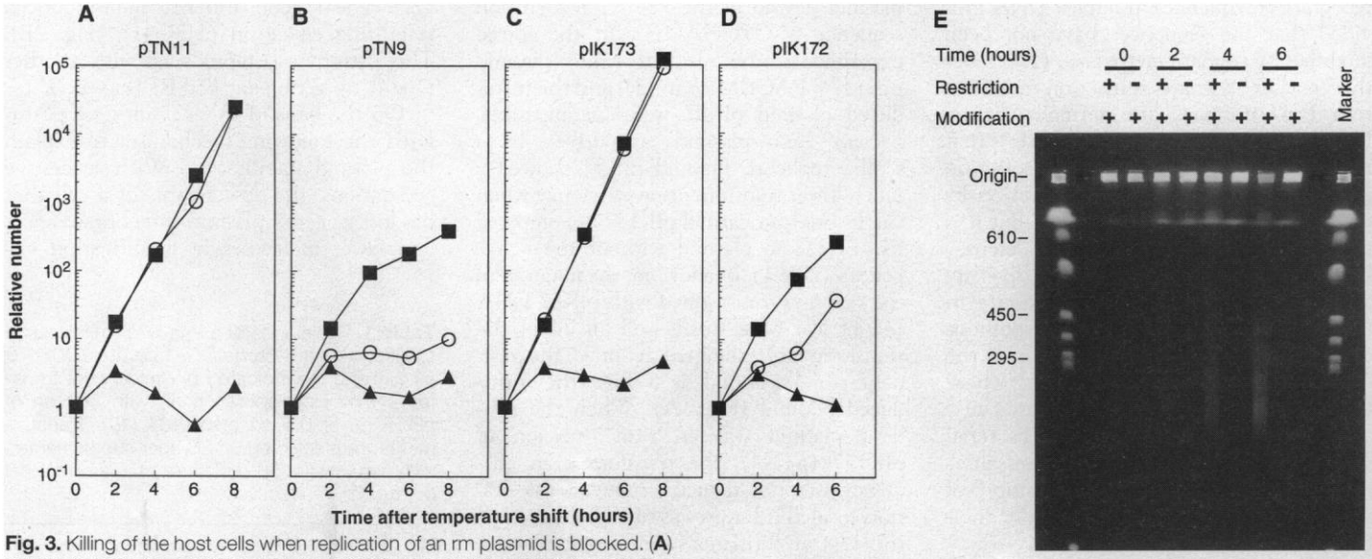

Fig. 3. Killing of the host cells when replication of an rm plasmid is blocked. (**A**) pTN11 (r^-m^+). (**B**) pTN9 (r^+m^+). (**C**) pIK173 (r^-m^+). (**D**) pIK172 (r^+m^+). The cells were aerated at 30°C in broth with ampicillin (50 μg/ml) until their optical density at 660 nm (OD_{660}) reached 0.3. The antibiotics were then removed and the temperature was increased to 42°C. The cells were diluted when the OD_{660} reached 0.3. The number of cells (■) was counted under a microscope. The number of viable cells (○) is the number of colonies on LB agar plates at 30°C. The agar was supplemented with ampicillin to measure the number of viable cells carrying the plasmid (▲). (**E**) Degradation of chromosomal DNA accompanying cell killing. In a culture of *E. coli* carrying pTN9 or pTN11, plasmid replication was blocked by a temperature increase from 30° to 42°C. The cells were treated with 2,4-dinitrophenol (final concentration 0.01%) after the indicated time intervals to block energy-dependent metabolism, especially DNA replication and adenosine triphosphate–dependent DNA degradation. DNA was prepared by an "in-agarose cell lysis" technique to minimize breakage (*13*) and was subjected to electrophoresis in a pulsed-field gel apparatus (Pharmacia) with hexagonal electrodes. Electrophoresis was performed at 165 V with a pulse time of 50 s for 24 hours. Marker lanes contain *Saccharomyces cerevisiae* chromosomes. Molecular sizes are indicated in kilobases.

cause of repair of the chromosomal double-strand breaks (5). Similar results were obtained with Eco RI (Fig. 3, C and D).

Cleavage of the host chromosome after plasmid loss was demonstrated directly by analysis of total DNA by pulsed-field gel electrophoresis (Fig. 3E). With the Pae R7 r^+m^+ plasmid, chromosome degradation was visible 2 hours after the temperature shift and became more extensive later, whereas no degradation was visible with the r^-m^+ control.

Gingeras and Brooks (3) cloned the Pae R7 r^+m^+ genes in pBR322 and showed that r^+m^- clones were both viable and stable and were phenotypically r^- during virus infection. To explain this apparent inconsistency with our results, we suggest that these earlier experiments were detecting an altered Pae R7 restriction enzyme activity, as the authors themselves hypothesized (3).

The same mechanism that ensures the stability of type II rm genes on a plasmid should also enforce the retention and functional integrity of the genes when they are chromosomally encoded. Failure to maintain the expression of an rm gene pair could kill the cell if the modification enzyme became sufficiently dilute before loss of effective restriction enzyme activity. Thus a pair of rm genes can be regarded as a parasite, symbiont, or "selfish" unit that enforces its retention in hosts whose chromosomes bear the specific sites it recognizes.

As an explanation for the widespread occurrence, diversity, and specificity of rm gene pairs, the "selfish gene" hypothesis is no less plausible than the "cellular defense" hypothesis. Both hypotheses are consistent with the finding that r and m genes are tightly linked and widely distributed. The diversity and high degree of specialization in sequence recognition, however, seem more readily explained as a consequence of adaptation to uncontested ecological niches, similar to competitive exclusion among species. In particular, the existence of rare cutter restriction enzymes is more consistent with the selfish gene hypothesis than with the cellular defense hypothesis.

Lethal behavior that affords survival value in the long run is fixed as programmed cell death during evolution. The mechanism of programmed cell death described here is formally similar to that of the post-segregational killing or addiction systems of plasmids and the postfertilization killing systems of maternal-effect selfish genes (6), although different in detail.

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Modulation of Chemotropism in the Developing Spinal Cord by Substance P

Carmen De Felipe, Robert D. Pinnock, Stephen P. Hunt*

Developing axons find their targets through direct contact with cues in the extracellular environment and in response to gradients of diffusible factors. The floor plate, a neuroepithelial structure, guides developing commissural axons in the spinal cord by release of chemoattractants. Floor plate cells express neurokinin-1 receptors, and a transiently appearing subpopulation of commissural axons contains substance P, the neuropeptide ligand for this receptor. Substance P increases the amount of axon outgrowth from dorsal horn explants cocultured with floor plate explants. Results of experiments with embryonic rats suggest that substance P released from pioneering neuronal pathways may regulate the release of chemoattractants from floor plate cells.

Diffusible factors released by cellular targets support and guide developing neurites. One example of this process occurs in the spinal cord, in which commissural axons are guided by factors released by the floor plate. The floor plate lies in the ventral midline of the neural tube. It is thought to have an early role in dorsoventral patterning of the neural tube and in the induction of motor neurons, and it is thought to become later an important intermediate target for developing commissural axons (*1*, *2*). In the rat, commissural axons originate from the dorsal neural tube, pass ventrally and medially to the motor neuronal column, and cross at the ventral midline, where they then turn sharply and adopt a rostral trajectory (*1*–*3*). In vitro it has been demonstrated that commissural fiber outgrowth from dorsal explants occurs only in the presence of the floor plate and that the orientation of neurite outgrowth from dorsal explants is crucially dependent on the relative position of the floor plate (*1*, *3*). We now present evidence that release of chemoattractants from the floor plate may be influenced by neuropeptides released from a subset of commissural neurons.

Using an antibody specific for the neurokinin-1 (NK1) receptor, the receptor for substance P, we have localized receptor protein throughout the rostrocaudal extent of the rat floor plate by embryonic day (E) 13 (Fig. 1A) (*4*). Immunoreactivity was seen only at rostral levels at E12 and was absent by postnatal day (P) 10. With immunofluorescence, we identified a group of sub-

C. De Felipe and S. P. Hunt, Division of Neurobiology, Laboratory of Molecular Biology, Medical Research Council, Hills Road, Cambridge CB2 2QH, UK.
R. D. Pinnock, Parke-Davis Neuroscience Research Unit, Forvie Site, Robinson Way, Cambridge CB2 2QB, UK.

*To whom correspondence should be addressed.